INTRODUCTION

Anastomotic leakage has been a problem in intestinal surgery for over a century. This may cause an increase in the need for reoperation, local risk recurrence, morbidity, and mortality rate, and also generally have a greater impact on quality of life. A study by Hammond et al. from 2008 until 2010 in the United States showed that anastomotic leakage increases financial expenses by 2.5 times higher compared to control. Of the various types of bowel anastomosis performed, the colon has the highest prevalence of anastomotic leak at 40%, followed by gastroduodenal anastomosis with a leakage rate of 32%.

It is known that anastomotic leakage can be influenced by various factors, both local and systemic factors. One example of influencing systemic factors is peritonitis, which disrupts the wound healing process. The wound-healing process plays a major role in the success of anastomosis, where leakage occurs when the healing phase in the anastomosis area is disrupted. The histopathological score is a semiquantitative method used to assess the wound healing process, which consists of collagen, fibroblast, and neovascularization density.

Several prevention methods have been developed in the last few decades to treat anastomotic leaks. One of them is the use of zinc and vitamin C mineral components which play a role in increasing the ability of the wound healing process. The role of zinc in the wound healing process can be seen from two perspectives. First, the impact of zinc deficiency, and second, the effect of zinc supplementation (topical, local or systemic) to a wound healing process. It is resolving zinc deficiency results in better wound healing. Apart from playing a role in the wound healing process, zinc is also useful in reducing the morbidity and mortality of patients with sepsis through the regulation of cytokines. Similarly with vitamin C, the significant impact of vitamin C on the wound healing process is mainly related to the synthesis and cross-linking of collagen, which contributes to vascular integrity and capillary strength. Dunphy’s study evaluating wounds in hamsters with vitamin C deficiency showed an association between vitamin C and the wound healing process, specifically in increased activity of fibroblasts and endothelial cells. Vitamin C supplementation promotes the catabolism of collagen formation. Redistribution and

ABSTRACT

Background: Colon has the highest risk of anastomotic leakage and increases 2.5-fold in peritonitis. To prevent leakage of colonic anastomosis, there are some modalities to upgrade the quality of wound healing. The mineral zinc is good for healing intestinal anastomotic wounds and vitamin C, whose function is related to wound healing and maintaining vascular integrity. This study investigated the effect of daily zinc and vitamin C supplementing on rabbits with peritonitis who underwent colonic anastomosis.

Material and methods: Thirty-six New Zealand rabbits underwent peritonitis 5 hours after they continued with a laparotomy to wash the abdominal cavity and colonic anastomosis. The rabbits were then divided into 2 groups. First group was given zinc and vitamin C syrup, while the second group was given normal saline without zinc and vitamin C. On the 6-day postoperative, the rabbits were terminated, and tissue was collected at the colonic anastomosis to examine the histological score of anastomotic wound healing.

Results: Significant differences in anastomotic healing in terms of histological scores (collagen density \( \alpha=0.00, \ p<0.05 \); Neovascularisation \( \alpha=0.00, \ p<0.05 \); Fibroblasts, \( \alpha=0.00, \ p<0.05 \)). This study generally explains the relationship between zinc and vitamin C administration and colonic anastomosis wound healing in peritonitis.

Conclusion: There were significant differences in histological wound healing scores (collagen density, neovascularization, fibroblasts) in the treatment group compared to the control group.

Keywords: Colon, Anastomotic, Peritonitis, Zinc, Vitamin C.

The sample size was calculated using the Federer replication formula with a minimum number of 16 for each group. Considering dropout caused by death, which may result in non-fulfilment of the minimum limit, we added 20%, which are 3 individuals from each group. Finally, there were 19 rabbits in the intervention group (zinc and vitamin C supplementation) and 19 rabbits in the control group (no zinc and vitamin C supplementation), with 38 rabbits. Enteral zinc supplementation was given at a dose of 10 mg/kg daily and vitamin C at 200 mg daily; both started post-surgery and continued for 5 days in treated animals. On the 6th day, the rabbits were terminated, and the anastomotic specimens 1.5 – 2 cm long were sent to the pathological anatomy department for histopathological examination of the total collagen density score, fibroblast count, and neovascularization.

The study was conducted within 7 months. The location of this research was carried out at the Research Centre of the Faculty of Veterinary Medicine (FKH) of Universitas Airlangga, while the manufacture of preparations was carried out at the Anatomical Pathology Laboratory, and for reading the results of the examination of preparations at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga. The data included as a result of the research was collected as primary data.

The data collected for the assessment of collagen density is in the form of ordinal data and will be compared between the group that was given post-laparotomy zinc and vitamin C supplementation and the group that was not given zinc and vitamin C supplementation. The hypothesis was tested with the non-parametric Mann-Whitney test. The number of fibroblasts from histological preparations was divided based on the groups that were given post-laparotomy zinc and vitamin C supplementation and those that were not. Subsequently, we calculated the lowest, highest, and average numbers in each group using an independent T-test.

RESULTS

Characteristics of Study Participants
This was an experimental study on 38 male white New Zealand rabbits Oryctolagus cuniculus aged 6–9 months 1 year of rabbit is equivalent with 7 years of human age) with body weight within range 2,000 – 2,800 g that is randomized into a control and intervention group each. This study aims to evaluate whether colonic anastomotic wound healing in peritonitis will improved with zinc and vitamin C supplementation compared to those without supplementation. Of the 38 samples, 36 samples are eligible to the research criteria and 2 rabbits dropped out because of death. The two rabbits died on the second day of acclimatization. No rabbits died during anesthesia, surgery, or at the 6th day of study. The death of the two rabbits was probably caused by stress due to the adjustment process to the environment. On the 6th day post-laparotomy, specimens were taken from the former colonic anastomosis and sent for histopathological examination.

Out of 36 samples in total, rabbits age ranged between 6 months and 9 months with a median age of 7 months in both groups. Mean age of intervention group is 7.052 ± 0.848 months and age of control group is 7.421 ± 0.707 months. Normality test was done with a result of abnormal distribution of age variable; therefore, Mann Whitney test was used. Furthermore, from the Mann-Whitney test, it is known that there is no statistically significant difference in the age distribution of the rabbits with p value of 0.284 (α=0.05).

Rabbit body weight was in the range of 2000 – 2800 g with an average of 2494.527 g with a standard deviation of 135.292 and 2484.210 g with a standard deviation of 195.115 g in the intervention group and control, respectively. The distribution data of body weight was normal; therefore, an independent sample t-test is used. From the independent sample t-test, there is no significant difference of body weight distribution between two groups with p value of 0.848 (α=0.05) (Table 1). Based on statistical calculations for the two groups, there was no significant difference in terms of body weight and age. All research rabbits were male (100%), thus the data was homogeneous in terms of gender.

Collagen Density in Anastomosis
Collagen density examination was carried out on 36 samples using a scoring system...
**Table 1.** Characteristic of participants.

<table>
<thead>
<tr>
<th>Demography</th>
<th>Control</th>
<th>Intervention</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>Range</td>
<td>6 – 9</td>
<td>6 – 9</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>7.421 ± 1.070</td>
<td>7.052 ± 0.848</td>
</tr>
<tr>
<td>Weight (gram)</td>
<td>Range</td>
<td>2000 – 2800</td>
<td>2000 – 2800</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>2484.210 ± 195.115</td>
<td>2494.527 ± 135.292</td>
</tr>
</tbody>
</table>

**Table 2.** Distribution data of collagen density score.

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Score 1</td>
<td>0 (0%)</td>
<td>10 (52.6%)</td>
<td></td>
</tr>
<tr>
<td>Score 2</td>
<td>1 (5.3%)</td>
<td>8 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>Score 3</td>
<td>11 (63.2%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Score 4</td>
<td>6 (31.6%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Distribution data of neovascularisation density score.

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Score 1</td>
<td>0 (0%)</td>
<td>7 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>Score 2</td>
<td>3 (15.8%)</td>
<td>12 (63.2%)</td>
<td></td>
</tr>
<tr>
<td>Score 3</td>
<td>16 (84.2%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.** Distribution data of number of fibroblast score.

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Score 1</td>
<td>0 (0%)</td>
<td>9 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>Score 2</td>
<td>1 (5.3%)</td>
<td>10 (52.6%)</td>
<td></td>
</tr>
<tr>
<td>Score 3</td>
<td>18 (94.7%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.** Histological distribution summary of wound healing.

<table>
<thead>
<tr>
<th>Wound healing</th>
<th>Control</th>
<th>Intervention</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Range</td>
<td>1 - 2</td>
<td>2 - 4</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>1.473 ± 0.512</td>
<td>3.263 ± 0.561</td>
</tr>
<tr>
<td>Neovascularisation</td>
<td>Range</td>
<td>1 - 2</td>
<td>2 - 3</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>1.631 ± 0.495</td>
<td>2.842 ± 0.374</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>Range</td>
<td>1 - 2</td>
<td>2 - 3</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>1.526 ± 0.512</td>
<td>2.947 ± 0.229</td>
</tr>
</tbody>
</table>

Neovascularisation in the Anastomosis

Neovascularization assessment was carried out on 36 using a scoring system of between 0 to 3, where a score of 0 indicates a lack of neovascularization, and conversely, a score of 3 indicates abundant neovascularization. There was a domination of scores 2 (15.8%) and 3 (84.2%) for the intervention group and a dominance of 1 (36.8%) and 2 (63.2%) for the control group. The median value of the neovascularization score was 2 in the treatment group and 3 in the control group, with an SD of 0.374 and 0.495 in the intervention and control groups, respectively, as described in table 3. The normality test of the neovascularization data revealed abnormal distribution; therefore, Mann Whitney statistical test was carried out. Mann-Whitney test revealed a significant difference in neovascularization between the group with zinc and vitamin C compared to the control group p-value of 0.000 (α=0.05) (Table 3). This suggested a significant difference between the neovascularization density score of the colonic anastomotic tissue that was given zinc and vitamin C supplementation and that which was not given zinc and vitamin C.

Fibroblast in the Anastomosis

Of the 36 research subject data, fibroblasts were evaluated and given score between 0 to 3, with a score of 0 indicating few fibroblasts to a score of 3 with many fibroblasts. The median value of fibroblast score was 1, which SD was 0.229 in treatment group and SD was 0.512 in control group. The normality test showed an abnormal distribution of fibroblast score data, so the Mann-Whitney statistical test was performed. The results of the Mann-Whitney test showed a significant difference in fibroblast density score of the colonic anastomotic tissue that received zinc and vitamin C supplementation and the control group that did not.
our study, peritonitis was induced in the experimental rabbits by injecting 10% autologous feces solution from 2 grams of feces collected from each rabbit dissolved in 20 ml of 0.9% NaCl, then 4 ml/kg of the solution was inserted into the intra-abdominal cavity. After 300 minutes or 5 hours, when the 10% autologous feces had been dissolved, colonic anastomosis laparotomy was carried out on the experimental animals with general anesthesia. Peritonitis was identified based on its symptoms, including lack of appetite and inactivity, and these conditions were evaluated together with the responsible veterinarian of our study. Furthermore, rabbits were administered a combination of vitamin C and zinc to evaluate the efficacy of the wound-healing process.

Colon resection and anastomosis are widely performed surgery in the field of colon cancer, devascularizing injuries, and infective pathology. Although this operation is generally safe, anastomotic leakage is a serious complication that can result in death. Several factors, including preoperative bowel preparation, surgical technique, nutritional status, and pathological intervention condition, significantly affect colonic anastomosis's healing. Despite the optimal use of surgical techniques and medical care, the integrity of the intestinal anastomosis may be compromised, resulting in wound dehiscence. According to a study in 2020 by Kothiya, it was stated that complications from non-optimal healing of anastomosis results in high both morbidity and mortality rates. Post-surgical zinc and vitamin C administration in this study was associated with colonic anastomotic wound healing quality in terms of collagen density, neovascularization, and the number of fibroblasts.

A study by Lansdown in 2007 proved that there was no significant difference in wound healing in experimental animals with normal zinc levels who received zinc supplementation compared to those without zinc supplementation, however in the setting of low level of zinc, zinc supplementation may result in a significant improvement of the wound healing compared to those who did not receive any zinc, where in this study peritonitis occurs, which theoretically results in decreased zinc concentration in the gastrointestinal tract. Alker and Haas in 2018 elucidate host defense mechanisms against pathogens. In septic conditions, TNF α and IL 6 cytokines will increase, indirectly producing concentrated zinc in the liver. This leads to a low zinc concentration in the periphery, including the gastrointestinal tract. Finally, this disrupts the wound-healing process in the colon. A study by Sutarja in 2021 also suggested a significant improvement in the healing process in the peritonitis of rabbits who underwent colon anastomosis with the administration of zinc supplementation compared to the group who did not receive any supplementation.

In 2019, Utomo, in the study, described that the administration of oral supplementation of zinc might increase blood concentration of zinc and improve the condition of sepsis in experimental animals, decreasing the risk of mortality and morbidity. The improvement in sepsis, according to the study by Utomo, results from a significant decrease in the level of the proinflammatory cytokine, such as TNF-α and IL 6, as already mentioned in the study by Alker and Haas in 2018. Similarly, Agren, in 2018, also found a significant difference in the healing process of the colonic anastomosis with a normal zinc level compared to the zinc-deficient group.

Previous study mentioned that repairing an anastomotic leak requires a long hospital stay, greatly increases costs, and often requires one or more repeat operations to control sepsis. Vitamin C is important in wound repair, healing, and regeneration processes because it stimulates collagen synthesis. Adequate vitamin C supplementation is necessary for the healing process, especially in postoperative patients, especially in jejunal and colonic anastomosis. Vitamin C is useful for rapidly synthesizing collagen at the wound site during the postoperative period. Satisfactory postoperative recovery is reflected in reduced plasma levels of proinflammatory and anti-inflammatory cytokines, which are higher in septic and surgical patients compared to healthy individuals. Circulating inflammatory cytokines reflect the general systemic inflammatory response and predict mortality in critically ill patients.

**DISCUSSION**

In this study, there was no bias found regarding age, gender, or body weight from all 36 experimental rabbits. New Zealand White rabbits are known have many good characteristics as experimental animals. Many researchers use rabbits aged 6-9 months as it is the age of maturity in rabbits, therefore, it is expected that the organs and developments are already mature. The range of body weight between 2000-3000 g was chosen based on the expected adequate nutrition for the associated age range, thus considered suitable as an experimental animal. In addition, our study samples have been declared healthy by veterinarian.

Peritonitis is a potentially life-threatening intra-abdominal pathological condition that partially manifests as an acute abdomen. Peritonitis affects about 9.3 patients per 1000 hospital admission. The diagnosis of acute peritonitis is essentially clinical and based on clinical features (abdominal pain, vomiting, impaired bowel transit, and abdominal contractures), which vary according to the underlying cause and duration of peritonitis progression.

Peritonitis is known to interfere with the healing process of anastomotic wounds. Peritonitis may induce altered expression of type I and III collagen genes and disrupts the synthesis of reparative collagen. These result in decreased concentration of minerals and vitamins that promote the wound healing process in the anastomosis. Fecal contamination and peritonitis significantly affect anastomosis healing of the colon by inhibiting collagen synthesis and the healing process. In this study, a model of rabbits with peritonitis was supplemented with zinc and vitamin C in the treatment group; subsequently, a comparative analysis of wound healing scores was carried out. In our study, peritonitis was induced in the control group with a p-value of 0.000 (α=0.05) (Table 4). It can be concluded statistically that there is a significant difference between the fibroblast density score of colonic anastomotic tissue that was given zinc and vitamin C supplementation and that which was not given zinc and vitamin C. All results were summarized in Table 5.

**Tabel 4**

**ORIGINAL ARTICLE**

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ill patients. In the systemic inflammatory response, inflammation and coagulation can be stimulated by microbial invasion (exogenous injury) or direct tissue injury (endogenous injury), when the NF-kB protein translocates into the nucleus and activates the transcription of both proinflammatory (e.g. IL-6) and anti-inflammatory cytokines, inflammation (eg IL-10). During inflammation, endothelial cells and polymorphonuclear leukocytes (PMNs) result in increased amounts of reactive oxygen species (ROS), which act as secondary mediators, inducing chemotactic substances (cytokines) and adhesion molecules that amplify the inflammatory process.\textsuperscript{18}

The wound healing process begins with an inflammatory phase which is influenced by the activation of platelets, neutrophils, and macrophages, whose levels increase until days 5 to 7 after the onset of the wound. Then in the proliferative phase, these factors will be replaced by wound healing growth factors, including fibroblast growth and increased neovascularization cellular. New fibroblast cells will appear towards the end of the inflammatory phase and the early stages of the proliferative phase. This proliferative stage is responsible for wound closure. Towards the end of the proliferative phase and the beginning of the remodelling phase, fibroblast cells and neovascularization will decrease, which will eventually be replaced by collagen and elastin biosynthesis.\textsuperscript{18}

Vitamin C is a water-soluble antioxidant molecule that acts as a ROS scavenger and has been shown to reduce cytokine production in various assays. Microcirculation is highly susceptible to oxidative stress leading to systemic inflammatory response syndrome, hemodynamic instability, and multiple organ failure. Plasma concentrations of vitamin C vary widely in patients with sepsis. Restoration of antioxidant and endothelial function in critically ill patients requires supraphysiological concentrations of ascorbate. Such concentrations can only be achieved by parenteral administration. Vitamin C is an important dietary antioxidant, which significantly reduces the detrimental effects of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA, and proteins. This additive effect reduces the adverse effects of peritonitis.\textsuperscript{19}

Vitamin C generally decreases the adverse effects of peritonitis by enhancing wound healing by increasing hydroxyproline content and improving tensile strength. Ascorbic acid supplementation has been shown to support intestinal myoelectric activity by exerting a neurotropic effect on ileal VIP-ergic neurons. In addition to the scavenging effect, ascorbic acid is obligatory in the hydroxylation of lysine and proline in collagen synthesis and cross-linking. Increased collagen production in the tissues contributes to the final strength of the wound. Studies with animal models of sepsis found that parenteral ascorbate enhances the survival, and healing of intestinal anastomosis and has beneficial effects on the cellular function of the innate and adaptive immune systems.\textsuperscript{20}

These works of literature support the theory that anastomotic wound healing is improved in experimental animals that received zinc and vitamin C supplementation in the setting of peritonitis in the treatment group compared to the control group.

The Efficacy of Postoperative Zinc and Vitamin C Supplementation on Increasing Collagen Density of Colon Anastomotic Wound in Peritonitis

Based on the results of this study, it was found that the collagen density was 50-75% in 12 treated rabbits, and 6 rabbits had a very dense collagen density. In the control group, 10-25% collagen density was found in 10 rabbits and 25-50% in 8 control rabbits. In this study, the collagen density was significantly better (p=0.000; α=0.05) in the zinc and vitamin C treatment group compared to the control group.

The Efficacy of Postoperative Zinc and Vitamin C Supplementation on Increasing Number of Fibroblast of Colon Anastomotic Wound in Peritonitis

In our study, it was found that there were significant differences in the number of fibroblasts. The increase in fibroblasts was higher and statistically significant (p=0.000; α=0.05) in the zinc and vitamin C treatment group compared to the control group. This is supported by research conducted by Hui lin et al., who reported that endothelial cells migrate and proliferate to the wound site along with re-epithelialization to form new blood vessels in a neovascularization process, or angiogenesis, thereby supplying oxygen and essential nutrients for cell growth at the base of the wound.锌 has been shown to be effective for in vivo angiogenesis.\textsuperscript{6}
Parenteral zinc supplementation increases re-epithelialization in a pig skin wound healing model. In theory, the increase in fibroblasts took place in the first 4 days. However, there was still a significant difference in this study on the 6th day after giving zinc and vitamin C, where a higher score was found in the intervention group. This illustrates the high inflammatory response in the intervention group while healing occurred. A study that tested Cu/zinc (Zn) galvanic microparticles reported that zinc was shown to increase keratinocyte migration and participate in the re-epithelialization of the epidermis.6

Regarding the distribution of collagen scores to the sample, 1 subject with a score of 2 in the treated rabbits and 2 subjects with a score of 2 in the control group, even though both had the same median score of 2. In the distribution of neovascularization scores, there were 3 subjects with a score of 2 in the treatment and 2 subjects with a score of 2 in control. Furthermore, in the calculation of fibroblasts, there was 1 subject with a score of 2 in the treatment group and 3 subjects with a score of 3 in the control group. These three phenomena indicate that various other possible factors affect the healing of anastomotic wounds. Several other factors may play a crucial role in the anastomotic healing process, however still potentially cause bias which has not been measured in our study, including the variation of route of drug administration, blood zinc levels (before peritonitis induction, before surgery, and 6 days after zinc and vitamin C administration), hemoglobin, albumin, tissue perfusion, and adequate oxygen delivery, postoperative stress, adequacy of postoperative nutritional input by experimental animals, as well as suture tension.

CONCLUSION
Zinc and vitamin C post-surgery significantly increase the collagen density, neovascularization, and the number of fibroblasts in the anastomosis colon wound during peritonitis.

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AUTHOR CONTRIBUTION
All authors contributed to the research and publication.

ETHIC CONSIDERATION
The local ethic commission has approved this research.

REFERENCES