IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ZINC SUPPLEMENTATION EFFECTS ON LAPAROTOMIC WOUND HEALING IN THE PERITONITIS CONDITION: A STUDY ON NEW ZEALAND RABBIT

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Abstract

Introduction: Laparotomy wounds in patients with peritonitis have a high risk of wound dehiscence. Wound healing disorders, often occur in peritonitis conditions, could result from a relative zinc deficiency in peritonitis conditions. Zinc concentrations in the blood of septic subjects were often found to be low due to peripheral redistribution of zinc to the liver.

Methods: This research was a randomized experimental research. A total of 36 male rabbits were induced to peritonitis by injecting 4ml/KgBW 10% autologous faecal solution and after six hours underwent exploratory laparotomy. The treatment group was then given zinc syrup supplementation of 10ml/KgBW per day for up to 5 days postoperatively. Wound healing was observed histopathologically with Hematoxylin Eosin and Masson Trichrome staining.

Results: More mature granulation tissue was found in the treatment group (mann whitney p=0.025), with higher collagen density (mann whitney p=0.039) and better re-epithelialization (mann whitney p=0.043). However, there was no significant difference between acute inflammation (mann whitney p=0.097), chronic inflammation (mann whitney p=0.507), granulation thickness (mann whitney p=0.735), and neovascularization (mann whitney p=1.0) in both groups. Further research would be needed to determine the mechanism by which zinc affects wound healing in peritonitis

Keywords: **zinc, peritonitis, wound healing.**

I. INTRODUCTION

Postoperative wound dehiscence has a significant impact on patients such as increased mortality, delayed discharge of patients, follow-up surgery, delayed administration of adjuvant drugs (in cancer patients), non-optimal wound cosmetics, and impaired psychosocial well-being of patients. An analysis of research data in America showed that patients with wound dehiscence experienced an increase in mortality by 9.6%, an additional length of hospitalization up to 9.4 days, and an additional bill of care up to USD 40,000 greater than controls (1). This wound dehiscence, although significant, is often underreported. According to the World Union of Wound Healing Societies 2018, the incidence of wound dehiscence in laparotomy surgery is 0.4%-3.8% but increases to 88% in contaminated and dirty operating conditions (1). The level of wound dehiscence in laparotomy surgery in the Pediatric Surgery Division of RSUD Dr. Soetomo reached 74 cases out of 1070 laparotomy operations (6.9%) in the 2014-2020 period. Increased awareness of the occurrence of wound dehiscence is needed, including prevention and management of wound dehiscence.

In the process of wound healing, macronutrients and micronutrients are needed in sufficient quantities to prevent disruption of the wound healing process. The micronutrient needed in almost every stage of wound healing is zinc (2). In the hemostasis phase, zinc plays a role in increasing platelet activity and aggregation which is regulated through Protein kinase C-mediated phosphorylation of platelet protein tyrosine (3). In the inflammatory phase, zinc plays a role in the recruitment and activation of non-specific immune cells to the wound site by inducing the release of alpha-granule platelets which contain a number of pro-inflammatory enzymes so as to initiate the inflammatory phase of wound healing (3). Zinc also affects the activity of several nonspecific immune cells such as

neutrophils, monocytes, macrophages, in the process of eliminating bacteria and damaged tissue, cytotoxicity, and apoptosis. The adaptive immune system that participates in the wound healing process is also influenced by zinc (2). In the proliferative phase, zinc plays a role in the migration of fibroblasts and keratinocytes to the wound tissue and increases angiogenesis and stem cell activation. In the remodeling phase, zinc plays a role in extracellular matrix remodeling and scar tissue formation (2).

Septic conditions, such as in patients with peritonitis, are known to reduce serum zinc levels by redistributing zinc into the liver (4). While increasing the need for zinc in the peritoneum (5). While in wound healing the need for zinc in the peripheral wound edges increases (2). The relative condition of peripheral zinc deficiency in this condition of peritonitis can interfere with the wound healing process. From previous studies, zinc supplementation in experimental animals with zinc deficiency can accelerate the wound healing process (6).

We're interested in examining whether the addition of oral zinc after laparotomy surgery performed under peritonitis conditions would improve wound healing or not. Oral zinc preparations were preferred over topical considering that the subjects in the study were in peritonitis conditions, the systemic zinc administrations were expected to improve the overall condition of the subjects simultaneously. The objectives in this study was to prove that the healing of the laparotomic wound in the sepsis condition would be better with zinc supplementation rather than without zinc supplementation.

II. Material and Methods

A total of 36 male Newzealand white rabbits aged 6-9 months with a weight of 2-3 kilograms were randomized into two treatments with 18 rabbits each, then adapted for seven days. Each rabbit was placed in a separate cage, with a 12/12 hour lighting cycle. Feeding and drinking during the adaptation period and ad libitum treatment.

After being adapted for seven days, the rabbits were made peritonitis by introducing 10% autologous feces solution, ie 2 grams of feces collected from each rabbit and dissolved in 20 ml of 0.9% NaCl, an amount of 4 ml/kgBW into the intra-abdominal cavity. After 6 hours from the injection, a laparotomy was performed under general anesthesia using ketamine 20-40 mg/kg body weight intramuscularly. During the induction of anesthesia, the therapeutic antibiotics Ceftriaxone 25 mg/kgBW and Metronidazole 10 mg/kgBW were also given (Badea and Santini, 2018). Each rabbit was shaved on the stomach, then disinfected with 10% povidone iodine and the operating field was narrowed with a sterile drape. A longitudinal longitudinal incision in the midline of the abdomen is 4 centimeters long, deepened to reach the peritoneum. Then the abdominal cavity was washed with a 0.9% NaCl solution of 300 ml/kgBW until clean. The laparotomy wound is then sutured. The layers of the peritoneum, fascia, and muscle were sutured simply with absorbable multifilament sutures (Polyglycolic Acid 4.0), while the skin was sutured continuously with non-absorbable monifalmen (Nylon 4.0) and the wound was closed with gauze.

Postoperatively, all rabbits were given a combination of advanced therapeutic antibiotics Ceftriaxone 25 mg/kgBW every 12 hours and Metronidazole 10 mg/kgBW every 8 hours, as well as analgesic Paracetamol at a dose of 10 mg/kgBW every 8 hours. Daily zinc supplementation was given to the treatment group at a dose of 10 mg/kgBW/day started after surgery and continued for 5 days. The rabbits were observed on day 6, at which time the rabbits were terminated to take the abdominal wall tissue from the laparotomy scar. If the rabbit dies before 6 days, it is considered a drop out.

Postoperative day 6, full-thickness abdominal wall specimens were taken from the laparotomy scar with cuts around the wound and 1 cm from the wound. Following fixation, the specimens then turned into paraffin section stained with hematoxylin eosin and Masson Trichrome and examined under a microscope at a magnification of 10-40x to assess wound healing based on the Abramov scoring system (8). To ensure reliability and validity, specimen readings were performed by an experienced pathologist in disguise (observers do not know the type of treatment of each specimen examined).

Comparisons between treatment and control group specimens were performed using the Mann-Whitney U non-parametric test. All statistical analyses were carried out using the SPSS version 13.0 data analysis system. p-Values < 0.05 were considered to be statistically significant for all comparisons.

III. Results

Four rabbits were dropped out because of death. Two rabbits, each from control and treatment group, died at two hours and three hours after peritonitis induction (before laparotomy). A rabbit from the control group died on the second post-operative day and a rabbit from the treatment group died on the fourth post-operative day. All rabbits that died after the procedure were evaluated for their intra-abdominal conditions and all of them found that their gastrointestinal organs were intact and there were no leaks. Their deaths were probably due to sepsis as a result of peritonitis. On the 5th post-laparotomy day, specimens were harvested from the laparotomic scar and sent for histopathological examination.

Of all rabbits, in the control and treatment groups, the weight range was between 2100-3100 grams with an average weight of the control group 2500+278.01 grams and the zinc group 2487.50+305.23 grams. Based on statistical calculations, it was found that the distribution of weight data was normally distributed, and the homogeneity test of the subjects with the parametric T-Test test of two independent samples obtained p value = 0.904 and = 0.05. While the age range in this study was 6-9 months with an average age of the control group 7.13 1.09 months and the zinc group 6.94+1.06 months. From the results of the normality test, it was found that the age distribution in this study was not normally distributed, and the results of the homogeneity test with the Mann-Whitney U non-parametric test obtained p values = 0.606 and = 0.05. Based on statistical calculations of the two groups, there were no significant differences in weight and age (table 3.1).

Table 3.1. Sample Characteristics Statics

Group		Zinc	Control	p-value*
Age .	N	16	16	.606
	Minimum	6	6	
	Maximum	9	9	
	Mean	6.94	7.13	
	Median	7.00	7.00	
	Std. Deviation	1.063	1.088	
	Normality Saphiro-Wilk (Significance)	.014	.04	
Weight	N	16	16	.904
	Minimum	2100	2100	
	Maximum	3100	3100	
	Mean	2487.50	2500.00	
	Median	2450.00	2500.00	
	Std. Deviation	305.232	278.089	
	Normality Saphiro-Wilk (Significance)	.377	.297	

***Homogen when p-value > 0,05**

3.1 Analysis of Wound Healing in Zinc Group Compared to Control

Wound healing in this study used a scoring system based on the modification of Abramov (1).

Table 3.2 Wound Dehiscence Results of Control and Zinc Group

Wound Healing Parameter		Control	Zinc
Collagen Density	Range	1	2
	Median ± SD	2 ± 0,479	2 ± 0,563
Neovascularization	Range	1	1
	Median ± SD	2,5 ± 0,516	2,5 ± 0,516
Acute Inflamation	Range	1	2
	Median ± SD	1 ± 0,512	1 ± 0,500
Chronic Inflammation	Range	2	2
	Median ± SD	1 ± 0,632	1 ± 0,619
Granulation Tissue Formation	Range	2	1
	Median ± SD	2,5 ± 0,719	2,5 ± 0,516
Granulation Tissue Maturity	Range	1	2
	Median ± SD	2± 0,5	2 ± 0,75
Re-epitelialization	Range	2	2
	Median ± SD	2 ± 0,806	2 ± 0,854

The scores of each group were analyzed statistically to be compared and assessed whether there was a significant difference between the two groups.

3.1.1 Analysis of Acute inflammation score

The acute inflammation score was assessed from the abundance of neutrophil cells and exudate of acute inflammatory cells with immature granulation tissue. Acute inflammation scores for both groups are shown in table 3.3.

Table 3.3 Acute Inflammation Scores Obtained in Each Group

Wound Healing Parameter	Score	Control	Zinc	p-value
Acute Inflammation	0	0	1	0,097
	1	9	12	
	2	7	3	
	3	0	0	
	Median	1	1	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

The Mann-Whitney test for acute inflammation scores of both groups resulted p value = 0.097, so it can be concluded statistically that there was no significant difference between acute inflammation scores of laparotomy wounds given zinc supplementation and those not given.

3.1.2 Analysis of Chronic inflammation score

The chronic inflammation score is determined by the number of plasma cells and monocytes that are evaluated. The percentages of the two groups as shown in table 3.4 were then analyzed using the Mann-Whitney test.

Table 3.4 Chronic Inflammation Scores Obtained in Each Group

Wound Healing Parameter	Score	Control	Zinc	p-value
Chronic Inflammation	0	0	0	0,507
	1	9	11	
	2	6	4	
	3	1	1	
	Median	1	1	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

The Mann-Whitney test for chronic inflammation scores of both groups resulted p value = 0.507, so it can be concluded statistically that there is no significant difference between chronic inflammation scores of laparotomy wounds given zinc supplementation and those not given.

3.1.3 Analysis of Granulation tissue formation score

The granulation tissue thickness score was determined from the number of fibroblast cells at the wound edges. The percentages of the two groups as shown in table 3.5 were then analyzed by the Mann-Whitney test.

Table 3.5 Granulation tissue formation score obtained in each group

Wound Healing Parameter	Score	Control	Zinc	p-value
Granulation tissue formation	0	0	0	0,735
	1	2	0	
	2	6	8	
	3	8	8	
	Median	1,5	2	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

The Mann-Whitney test for granulation tissue formation scores for both groups resulted p value = 0.735, so it can be concluded statistically that there is no significant difference between the scores of granulation tissue thickness in laparotomy wounds given zinc supplementation and those not given.

3.1.4 Analysis of Granulation tissue maturity score

Granulation tissue maturity score is determined from the shape and regularity of the arrangement of fibroblast cells at the wound edges. Mature fibroblast cells are flat and arranged in parallel in dense layers, while immature fibroblasts are stellate and irregularly arranged. The percentages of the two groups as shown in table 3.6 and then analyzed by the Mann-Whitney test.

Table 3.6 Granulation tissue maturity score obtained in each group

Wound Healing Parameter	Score	Control	Zinc	p-value
Granulation tissue maturity	0	0	0	0,025
	1	6	3	
	2	10	7	
	3	0	6	
	Median	2	2	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

The Mann-Whitney test for granulation tissue maturity scores for both groups resulted p value = 0.025, so it can be concluded statistically that there was a significant difference between the treatment group and control. Comparison of granulation tissue maturity scores between the two groups were shown in Figure 3.1.

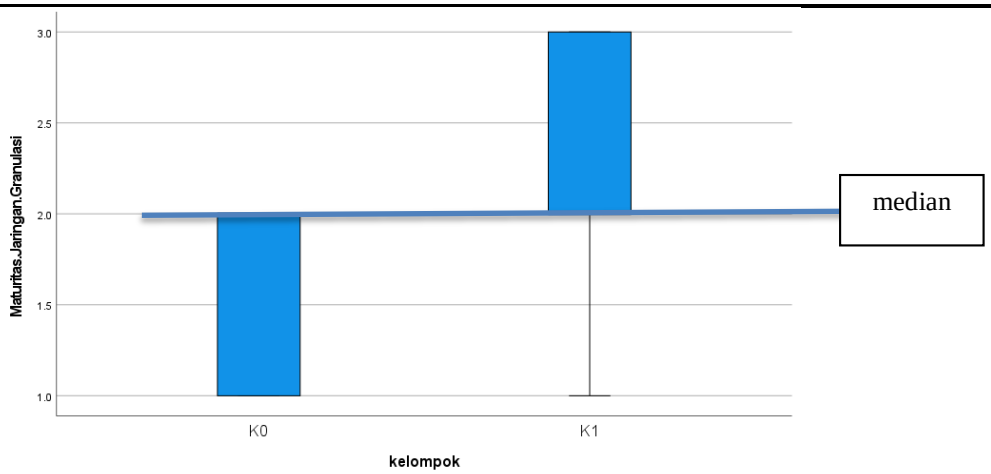

Figure 3.1 Comparison of Granulation Tissue Maturity Score K0 (control group) and K1 (zinc treatment group)

3.1.5 Analysis Neovascularization score

The neovascularization score is determined by the amount of neovascularization that forms at the wound margin. The percentages of the two groups as shown in table 3.7 were then analyzed using the Mann-Whitney test.

Table 3.7 Neovascularization Score obtained in each group

Wound Healing Parameter	Score	Control	Zinc	p-value
Neovascularization	0	0	0	1,0
	1	0	0	
	2	8	8	
	3	8	8	
	Median	2,5	2,5	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

get p value = 1.00 while = 0.05, so it can be concluded statistically that there is no significant difference between the neovascularization scores of laparotomy wounds given zinc supplementation and those not given.

3.1.6 Analysis Re-epithelialization score

The re-epithelialization score was determined from the thickness of the epithelium at the wound edges. The percentages of the two groups as shown in table 3.8 were then analyzed using the Mann-Whitney test.

Table 3.8 Neovascularization Score obtained in each group

Wound Healing Parameter	Score	Control	Zinc	p-value
Re-epithelialization	0	3	0	0,043
	1	4	5	
	2	9	5	
	3	0	6	
	Median	2	2	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

Mann-Whitney test for re-epithelialization scores for both groups resulted p = 0.043, so it can be concluded statistically that there was a significant difference between the scores for re-epithelialization of laparotomy wounds given zinc supplementation and those not given. The comparison graph of re-epithelialization scores between the two groups is depicted in Figure 3.2.

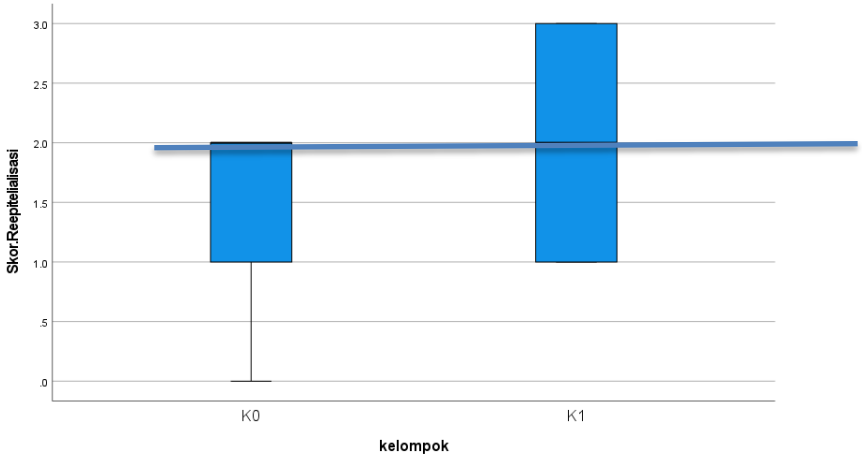

Figure 3.2 Comparison of re-epithelialization score between K0 (control group) and K1 (zinc treatment group)

3.1.7 Analysis of collagen deposition score

Collagen deposition score was determined from the thickness of the collagen fibers at the wound edges as indicated by the blue colored fibers on the tissue preparations with Masson Trichrome staining. The percentages of the two groups are shown in table 3.9 and then analyzed by the Mann-Whitney test.

Table 3.8 Neovascularization Score obtained in each group

Penyembuhan Luka	Skor	Kontrol	Zinc	p-value
Deposisi Kolagen	0	0	0	0,039
	1	5	3	
	2	11	7	
	3	0	6	
	Median	2	2	

* the test using Mann Whitney and considered to be significant/meaningful if the p-value <0.05

The results of the Mann-Whitney test for collagen deposition scores of both groups got p=0.039 while =0.05, so it can be concluded statistically that there is a significant difference between the collagen deposition scores of laparotomy wounds given zinc supplementation and those not given. The graph of the comparison of collagen deposition scores between the two groups is shown in Figure 3.3.

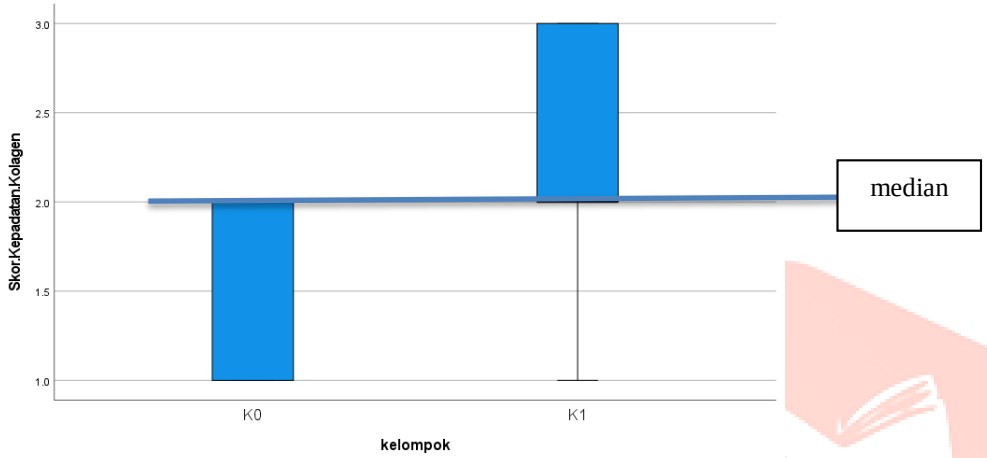

Figure 3.3 Comparison of collagen deposition score between K0 (control group) and K1 (zinc treatment group)

IV. DISCUSSION

In this study, peritonitis condition was induced to the healthy rabbits population that were subsequently randomized to be given zinc supplementation. Oral zinc preparations were preferred over topical considering that the subjects in the study were in peritonitis conditions, the systemic zinc administrations were expected to improve the overall condition of the subjects simultaneously. Although the toxicity of zinc is said to be quite low, from a study it is known that the effect of zinc supplementation depends on the dose of zinc given. Doses above 30 mg/kgbB were said to have a pro-inflammatory effect (2). It could prolong wound healing. In this study, we used a dose of 10 mg/kgBW to avoid the proinflammatory effect of zinc.(3)

Four rabbits died in this study. From the inspection of the rabbit that died after laparotomy, there was no intestinal perforation in the abdominal cavity. So that the death of the rabbits was most likely due to the sepsis condition they experienced.

There was no significant difference between acute and chronic inflammation scores, which could be due to the fact that the two groups had entered a further healing phase so that there was no significant difference in markers of the inflammatory phase of the two groups. This is in accordance with research from Abramov (1) which found the peak of acute and chronic inflammation in the abdominal wall of rabbits was on the 4th postoperative day.

Regarding granulation tissue thickness scores and neovascularization scores, there were no statistically significant differences, it could be because the treatment and control groups were both in a more advanced wound healing phase. According to Abramov's study, the thickness of granulation tissue and neovascularization increased to a peak on day 4 and then decreased thereafter (1).

Another possibility that cannot be ruled out is that oral zinc administration did not have an impact on inflammation, granulation tissue thickness, and neovascularization. This will be explained by further research on the effect of zinc on pro-inflammatory and anti-inflammatory enzymes in the early stages of wound healing in peritonitis.

There are three scores that indicate a significant difference between the two groups, namely the granulation tissue maturity score, reepithelialization score, and collagen density score. According to previous studies, granulation tissue maturity will increase steadily from day 4 to day 35 after injury. Similarly, reepithelialization and collagen deposition increased steadily from day 4 to 35 after injury (1).

The significant differences in granulation tissue maturity, reepithelialization, and collagen density indicated that zinc administration improved the proliferative phase of laparotomy wounds in peritoninitis. One of the reasons for the improvement in wound healing in this study could be the increase in the availability of zinc in the periphery so that it meets the needs of the enzymes used to improve wound healing. Wound healing enzymes associated with zinc include alkaline phosphatase, RNA and DNA polymerase, and Matrix Metalloproteinase (4). Alkaline phosphatase is an early marker of angiogenesis and smooth blood vessels associated with increased inflammatory activity and connective tissue proliferation (4). DNA polymerase is an accurate marker of cell proliferation. Meanwhile, MMP has a proteolytic function in wound debridement and plays a role in regulating cell migration

and extracellular matrix replacement. In the administration of MMP inhibitors which block all proteolytic and collagenolytic functions of MMP, there is disruption of keratinocyte migration and wound contraction during the wound healing process (5).

The high availability of zinc in the periphery can first be caused by differences in the initial conditions of zinc concentrations in the periphery before the study began. The next possible cause is the presence of zinc supplementation or due to improvement in the subject's systemic condition which causes a decrease in proinflammatory enzymes, allowing a shift of zinc from the liver back to the periphery.

One of the factors that can influence the impact of oral zinc supplementation is the process of zinc transport from the gastrointestinal tract to the wound edges. Zinc is transported out of the basolateral membrane of intestinal epithelial cells into the mesenteric circulation where it binds to plasma proteins, especially albumin. The protein-bound zinc is then transported through the portal circulation to the liver, where it is taken up and released and then distributed to other tissues. In serum, zinc is mainly bound to albumin (about 85%), to a lesser extent to a2-macroglobulin (about 16%) and amino acids (1% to 2%) (6). Changes in circulating concentrations of these proteins, particularly albumin, can dramatically alter plasma zinc levels.

Under conditions of peritonitis, there may be a decrease in plasma albumin (7), a condition that has the potential to interfere with zinc transport in the body. In addition, in conditions of peritonitis zinc undergoes redistribution from serum to the liver (8). The presence of entero-hepatic circulation which is involved in the process of zinc metabolism through the oral route makes circulating levels of zinc in the blood much lower than that given. These three factors further sparked the idea that there are other mechanisms of action of zinc that influence wound healing in peritonitis. Different results will be obtained when zinc supplementation is given via injection.

The possibility of wound repair in the treatment group in this study could be due to because zinc suppresses pro-inflammatory conditions that occur during sepsis so that it can improve tissue repair function by the body. Several animal studies have shown that zinc supplementation in sepsis causes a decrease in the pro-inflammatory cytokines IL-6, IL-8, and TNF- as well as an increase in the anti-inflammatory cytokines IL-10 and TGF-β (9). Studies in neonates with neonatal sepsis have shown that zinc supplementation improves outcomes and reduces mortality (10).

By decreasing pro-inflammatory enzymes, in turn, it can restore the distribution of zinc from the liver to the periphery including wound edges so that the need for zinc for wound healing can be met.

The drawback of this study is that not all subjects (subjects who dropped out or died or who died before the end of the study were not all underwent laparotomy. Likewise, measurements of Hb, zinc, and other indicators were not carried out before and after treatment. Measurement of zinc levels before and after treatment after peritonitis occurs, and measurement of zinc levels at the edges of the wound and in the liver after zinc supplementation will further explain the mechanism of zinc in influencing wound healing in peritonitis conditions..

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