

Basic Medical Research

The effect of ascorbic acid irrigation during debridement on the colony count of staphylococcus aureus and interleukin-6 blood count in grade II open fracture of long bone

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ABSTRACT

ABSTRAK

Introduction: Infection in open fractures is still a problem that can not be fully managed. Various types of studies have been conducted to find an effective and efficient solution to wash open fracture wounds. This study analyzed the effectiveness of ascorbic acid irrigation in reducing Staphylococcus aureus colony and IL-6 in open fractures grade II.

Methods: This study was an experimental study with pre- and post-test control group design. In this study, the colony count of Staphylococcus aureus and IL-6 in open fracture was treated with additional irrigation of 10mg/mL ascorbic acid during debridement. This study included 24 subjects with Gustillo type II open fractures, which were divided into 2 groups.

Results: Statistical analysis using Wilcoxon test showed that there was no significant difference ($p > 0.05$) in the reduction of Staphylococcus aureus colonies ($p = 0.308$) and IL-6 ($p = 0.239$) blood count between the Control Group and the Treatment Group.

Conclusion: Ascorbic acid irrigation did not decrease Staphylococcus aureus colony and IL-6 blood count in open fracture grade II of long bone.

Pendahuluan: Infeksi pada fraktur terbuka masih merupakan masalah yang belum dapat ditanggulangi sepenuhnya. Berbagai jenis penelitian telah dilakukan untuk mencari larutan yang efektif dan efisien untuk pencucian luka. Penelitian ini menilai efek pemberian irigasi asam askorbat pada saat debridement terhadap koloni staphylococcus aureus dan kadar IL-6 darah pada pasien dengan fraktur terbuka tulang panjang grade II.

Metode: Penelitian ini merupakan penelitian eksperimental dengan rancangan studi pre-post test control group design. Penelitian ini membandingkan jumlah koloni Staphylococcus aureus dan kadar IL-6 darah pada kelompok fraktur terbuka dengan irigasi larutan asam askorbat 10mg/mL pada saat debridement. Penelitian ini mengikut sertakan 24 subyek penelitian dengan fraktur terbuka tipe II menurut Gustillo yang dibagi menjadi 2 kelompok.

Hasil: Analisis statistik menggunakan uji Wilcoxon menunjukkan bahwa tidak ada perbedaan yang signifikan ($p > 0,05$) dalam penurunan jumlah koloni Staphylococcus aureus ($p = 0,308$) dan kadar darah IL-6 ($p = 0,239$) antara kelompok kontrol dan kelompok perawatan.

Kesimpulan: Irigasi asam askorbat tidak menurunkan koloni Staphylococcus aureus dan jumlah darah IL-6 pada fraktur terbuka tulang panjang grade II.

Keywords: Open fracture, Staphylococcus aureus, IL-6, ascorbic acid

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INTRODUCTION

Open fracture is one of the emergencies in Orthopedics and requires emergency treatment. Open fractures occur in around 3-4% of all fractures.¹ Infection is one of the major complications in open fracture, with the incidence of positive bacteria cultures of 70% - 80%.^{2,3} The most commonly found bacteria are gram-positive bacteria, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.^{1,4,5}

The purpose of open fracture management is to reduce the number of infections, including administration of antibiotic and anti-tetanus prophylaxis, debridement and irrigation, fracture stabilization and wound closure.^{6,7,8,9}

There are various controversies in open fracture management, including the use of a number of types of irrigation, the choice of antibiotics, the timing of surgery and wound closure. A study by Hadiwidjaja (2015) showed that additional washing using antibiotic solutions and antiseptic solutions in open fractures after debridement and internal fixation did not provide additional benefits in terms of reducing the number of colonies of *Staphylococcus aureus* compared to saline solution.¹⁰ Irrigation with bacitracin solution or mixed with soap did not give any difference in the number of different infections. The administration of various isotonic liquids (distilled water, boiled water, and tap water) also did not provide significant results.^{8,9}

Based on Suslovitch's study in 2015, *in vitro* ascorbic acid administration on *Staphylococcus aureus* agar plates inhibited the growth of *Staphylococcus aureus* and formed a zone of inhibition on the plate so that *Staphylococcus aureus*'s area was restricted to 2-4 mm.¹¹ Research by Fu et al., 2013 proved that the use of vitamin C irrigation in ACL reconstruction could reduce the blood CRP level and promote healing compared to normal saline irrigation.¹² In one study, 1g of ascorbic acid significantly treated and reduced inflammation, as measured with hs-CRP and IL-6 in adults with diabetes mellitus.^{13,14}

This study evaluated the use of ascorbic acid irrigation during debridement in open fractures. This study also evaluated the effect of post-debridement irrigation using ascorbic acid in reducing colonies of *Staphylococcus aureus* and in reducing the systemic inflammatory response (IL-6 blood count).

METHODS

This study was an experimental study with pre- and post-test control group design. This study analyzed the colony of *Staphylococcus aureus* and IL-6 blood count in open fracture with additional irrigation of 10mg/mL ascorbic acid during debridement.

This research was conducted at the Department of Orthopaedics and Traumatology, Sanglah Hospital/ Faculty of Medicine, Udayana University, Denpasar. The examination of culture samples was carried out at the Microbiology Department of Sanglah General Hospital, Denpasar. IL-6 examination was carried out at the Clinical Pathology Department, Sanglah General Hospital, Denpasar.

Samples of this study were patients with open fractures who came to Surgical Emergency Installation in Sanglah General Hospital, Denpasar, and met the inclusion criteria and willing to participate in this study. The number of samples for each group was 12 subjects. Therefore, the total number of samples needed in this study were 24 subjects.

In this study, the samples were divided into 2 groups, Control Group and Treatment Group. Bacterial culture samples and IL-6 blood count were taken pre-operatively from the patients with grade II open fracture who fulfilled the criteria. Afterwards, in the Control Group, debridement and irrigation were carried out using normal saline, whereas in the Treatment Group 50 mL of 10g/mL ascorbic acid was added to the irrigation. Postoperative culture was taken from both groups and blood was collected for IL-6 blood count analysis at 24 hours postoperatively.

RESULTS

This study consisted of 24 patients as samples, 18 (75%) were male patients and 6 (25%) were female patients, with age ranging from 17 to 66 years and the mean age of 32.58 years (Figure 1 and Figure 2). Based on the location of the open fracture, this study consisted of 2 (8.3%) patients with open humeral fractures, 2 (8.3%) patients with open fractures in forearm, 7 (29.2%) patients with open fractures in the femur, and 13 (54.2%) patients with open fractures in cruris. (Table 1)

Data of *Staphylococcus aureus* colony in the Control

and Treatment Groups was analyzed descriptively. In the control group, colony of *Staphylococcus aureus* pre- and post-debridement were 15021.58 CFU/cm² and 1507.91CFU/cm², respectively. In the Treatment Group with ascorbic acid irrigation, colony of *Staphylococcus aureus* pre- and post-debridement were 1037 CFU/cm² and 155.16 CFU/cm², respectively (Table 2).

descriptively. The decrease in *Staphylococcus aureus* colonies in the Control and the Treatment Groups were 13513.66 CFU/cm² and 881.33 CFU/cm², respectively (Table 3).

Table 2. Descriptive analysis of Staphylococcus aureus colony pre- and post-treatment

Group		Colony (CFU/cm ²)	
		Pre	Post
Control	Mean	15021.58	1507.91
	Lowest	10	0
	Highest	80566	12250
Treatment	Mean	1037	155.16
	Lowest	10	0
	Highest	6155	1240

Table 3. Descriptive analysis of the decrease in the number of Staphylococcus aureus colonies

Group		Colony (CFU/cm ²)
Control	Mean	13513.66
Treatment	Mean	881.33

The Wilcoxon test resulted in a decrease in *Staphylococcus aureus* colonies with p = 0.308 (p > 0.05). This result showed that the reduction in *Staphylococcus aureus* colonies was not significant between the Control and the Treatment Group (Table 4).

Data of IL-6 blood count in the Control and the Treatment Groups was analyzed descriptively. In the control group, IL-6 blood count pre- and post-debridement were 61.17pg/mL and 11.37pg/mL, respectively. In the Treatment Group, treated with ascorbic acid irrigation, IL-6 blood count pre- and post-debridement were 89.65pg/mL and 97.63pg/mL, respectively (Table 5).

Data on the decrease in the amount of IL-6 blood count was obtained by calculating the difference in the number of colonies before and after debridement. The data was subsequently grouped according to the Control and the Treatment Groups and analyzed descriptively. The decrease in IL-6 blood count in the Control and the Treatment Groups were 23.97pg/mL and 7.59pg/mL, respectively (Table 6).

The Wilcoxon test resulted in a decrease in IL6 level with p = 0.239 (p > 0.05). Thus, the IL-6 blood count was found to be not significantly different between the

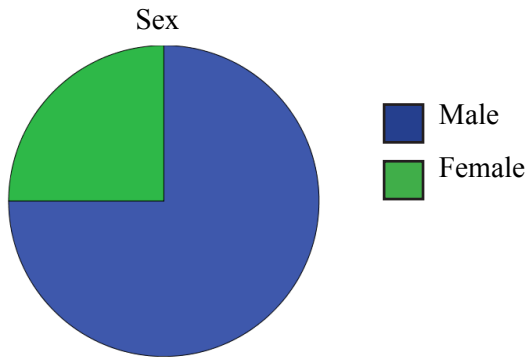


Figure 1. Sex descriptive analysis

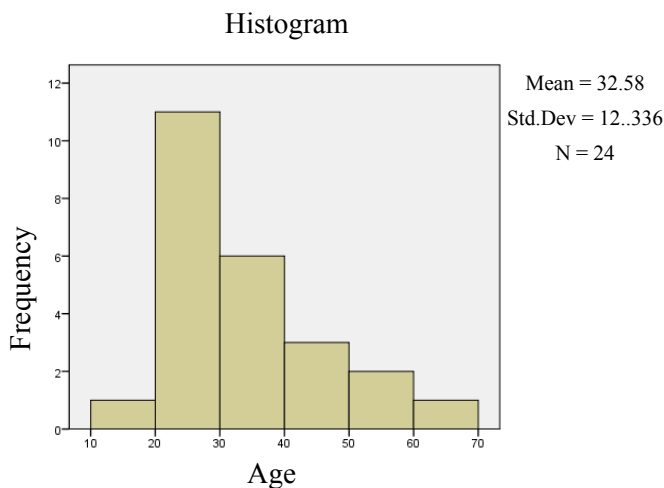


Figure 2. Age descriptive analysis

Table 1. Descriptive analysis of open fracture location

Location	N	Percentage (%)
Humerus	2	8.3
Antebrachii	2	8.3
Femur	7	29.2
Cruris	13	54.2
Total	24	100

The data on the decrease in the number of colonies of *Staphylococcus aureus* was obtained by calculating the difference in the number of colonies before and after debridement. Next, the data were grouped according to the Control and the Treatment Groups and analyzed

Control Group and the Treatment Group (Table 7).

Table 4. Wilcoxon Test of the decrease in Staphylococcus aureus colony

Test Statistics ^b	
SAvitC - SAKontrol	
Z	-1.020a
Asymp. Sig. (2-tailed)	.308

a. Based on positive ranks.
b. Wilcoxon Signed Rank Test

Table 5. Descriptive analysis of IL-6 blood count pre- and post-treatment

Group		pg/mL	
		Pre	Post
Control	Mean	61.17	11.37
	Lowest	6.5	379.88
	Highest	218.91	85.15
Treatment	Mean	89.65	97.63
	Lowest	7.59	10.08
	Highest	369.88	393.11

Table 6. Descriptive analysis of increase in IL-6 blood count

Group	(pg/mL)
Control	Mean 23.97
Treatment	Mean 7.59

Table 7. Wilcoxon Test of the decrease in IL-6 blood count

Test Statistics ^b	
IL6vitC - IL6kontrol	
Z	-1.177a
Asymp. Sig. (2-tailed)	.239

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

DISCUSSION

Analysis of the effectiveness of ascorbic acid irrigation post-debridement used *Staphylococcus aureus* colony count because *Staphylococcus aureus* is the most common micro organism found in open fractures. Ascorbic acid irrigation was done to decrease post-debridement contamination and to decrease the risk of infection. One recent study showed that *in vitro* ascorbic acid administration formed 2-4 mm inhibition zone around

the *Staphylococcus aureus* colony on an agar plate.¹¹ Another study showed that ascorbic acid incorporated in the irrigation in ACL reconstruction could decrease CRP level and promote better healing compared to normal saline irrigation.¹²

The decrease in the number of colonies of *Staphylococcus aureus* showed a difference between pre- and post-debridement, but based on the results of statistical analysis, irrigation of ascorbic acid did not provide additional benefit in reducing the number of contaminant in type II open fractures according to Gustillo. Several factors that can cause these results include:

- The degree of initial contamination cannot be fully controlled. This is indicated by a significant difference in the number of *Staphylococcus aureus* colonies before treatment.
- Open fractures in the upper extremities have a lower tendency to contamination than the lower extremities. This study does not distinguish the location of open fractures, so that variations can occur in terms of the degree of initial contamination.
- This study does not differentiate the period in hours. So that the patients who get treatment at the first hour are assumed to be the same as patients who get treatment at the sixth hour.
- Sampling was only done before the debridement and after the treatment. So, in this study, it was not possible to distinguish at what stage the decrease in the number of *Staphylococcus aureus* occurred.
- In this study a dose of 50mL of 10mg/mL ascorbic acid was used, which has been shown to help accelerate soft tissue healing, but this dose is not effective enough to reduce the colonies of *Staphylococcus aureus*.

Evaluation of IL-6 blood count was done to see the effect of ascorbic acid irrigation in inflammation process. IL-6 is a pro-inflammatory cytokine that will increase in open fracture. Previous study showed that ascorbic acid could decrease inflammation significantly as measured with hs-CRP level and IL-6 level in diabetic patient.¹³

Ascorbic acid administration in irrigation has never been done before, so in this study the dosage of ascorbic acid irrigation was based on the research by Fu et al., 2013 that used 50 mL of 10 mg/mL ascorbic acid irrigation in ACL reconstruction. In this study, ascorbic acid irrigation in ACL reconstruction could decrease CRP

level and promoted better healing than normal saline irrigation.¹² Ascorbic acid in inflammation process will modulate DNA that bind nuclear factor-kappa B (NF- κ B). Inflammation is induced by oxidative stress and will then induce cytokine and molecule adhesion in vascular endothelial that finally will produce TNF- α and IL-6 by the liver. Ascorbic acid can reduce the inflammatory mediator, TNF- α and IL-6, by decreasing the expression of hepatic mRNA.¹³ Ascorbic acid delay the activation of TNF- α of human NF- κ B in vitro and decrease the production of GM-CSF, IL-3, and IL-5.¹⁵

The analysis of the effect of ascorbic acid treatment in the Control and the Treatment Groups was done by comparing the difference in the mean value of reduction in IL-6 blood count. The Wilcoxon test resulted in a decrease in IL6 level with $p = 0.239$ ($p > 0.05$). Thus, the difference in IL-6 blood count was found to be not significant between the Control Group and the Treatment Group. The difference in IL-6 blood count that is not significant in this study can be caused by:

- The dose and volume of ascorbic acid solution used are inadequate to provide anti-inflammatory effect considering that the dosage used is the dosage used in arthroscopy for soft tissue healing.
- Contamination and different degree of damage to soft tissue cause different elevation of IL-6 blood count, resulting in bias in this study.
- Sampling was only done before the debridement and 24 hours after the treatment so that it could not be observed at what stage the increase in IL-6 blood count occurred and it was not possible to see how the IL-6 blood count decreased after 24 hours.

CONCLUSION

Based on this study, it can be concluded that irrigation using ascorbic acid during debridement does not reduce the number of colonies of *Staphylococcus aureus* and IL-6 blood count in grade II open fractures of long bones.

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