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Original Research Article

Ascorbic acid irrigation during debridement decreased the colony count of Staphylococcus aureus and interleukin-6 blood count in grade ii open fracture of long bones

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ABSTRACT

Background: Infection in open fractures is still a problem that cannot be fully managed. Various types of studies have been conducted to find an effective and efficient solution to wash open fracture wounds. This study compared the effectiveness of ascorbic acid irrigation in reduction of *Staphylococcus aureus* colony and interleukin-6 (IL-6) serum level as systemic inflammation marker in debridement procedure of grade II Gustilo-Anderson open fracture of long bones.
Methods: This study was an experimental study with pre-post-test control group design. This study included 24 subjects aged 15-75 years old with Gustillo-Anderson type II open fractures which were divided into two groups. The treatment group had additional irrigation by using 10 mg/ml ascorbic acid solution during debridement, whereas the control group had debridement only without irrigation with ascorbic acid. The culture and colony count of *Staphylococcus aureus* and IL-6 serum level was obtained and measured before and after the treatment in both groups. Statistical analysis (Wilcoxon test) was performed to compare the difference of the two parameters (Δ colony count and Δ IL-6) before and after the treatment.
Results: There were no significant difference in Δ colony count of *Staphylococcus aureus* (p=0.308) and ΔIL-6 serum level (p=0.239) between the control group and treatment group.
Conclusions: Ascorbic acid irrigation was not proved to decrease the colony count of *Staphylococcus aureus* and IL-6 serum level in grade II open fracture of long bones.

Keywords: Ascorbic acid, Interleukin-6, Open fractures, *Staphylococcus aureus*

INTRODUCTION

Open fracture is one of the emergencies in orthopaedics and requires emergency treatment. Open fractures occur around 3-4% of all fractures.[1] Infection is one of the major complications in open fracture, with the incidence of positive bacteria cultures 70-80%.[2,3] The most commonly found 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, include administration of antibiotic and anti-tetanus prophylaxis, debridement and irrigation, fracture stabilization and wound closure.[6-9] There is various controversies in open fracture management including the use of the number and type of irrigation, the choice of antibiotics, the timing of surgery and wound closure. Based on Suslovitch's study in 2015, in vitro ascorbic acid administration on *Staphylococcus aureus*

agar plates, ascorbic acid inhibits the growth of *Staphylococcus aureus* and forms a zone of inhibition on the plate so that *Staphylococcus aureus's* area is restricted to 2-4 mm.[10] Research by Fu et al proved that the use of irrigated vitamin C in anterior cruciate ligament (ACL) reconstruction can reduce blood C-reactive protein (CRP) levels and promote healing compared with irrigation using normal saline.[11] In one study, the effect of 1 gm ascorbic acid significantly treated and reduced inflammation, as measured by high-sensitivity-CRP (hs-CRP) and IL-6 in adults with diabetes mellitus.[12,13]

This study evaluates the use of ascorbic acid irrigation during debridement in open fractures. The researcher will evaluate the effect of post debridement irrigation using ascorbic acid in reducing colonies of *Staphylococcus aureus* bacteria and reducing the systemic inflammatory response (presented by IL-6 serum level).

METHODS

This study was an experimental study with pre-post-test control group design. The population was patients with Gustillo-Anderson type II open fractures of long bones. This study included patients aged 15-75 years old with Gustillo-Anderson type II open fractures in long bones (defined as open fracture of humerus, radius, ulna, femur, tibia, and fibula, moderately communitive, with wound sized less than 1 cm without extensive soft tissue in injury, flapping, or avulsion) who came to Surgical Emergency Department in Sanglah General Hospital Denpasar between the period of May 2018 until November 2018. The exclusion criteria were patients with any comorbid so surgical treatment cannot be performed, multiple fractures, negative result of pre-treatment culture, and those who refused to participate in the study.

The samples were obtained by consecutive sampling and then were divided into two groups (12 subjects per group). The treatment group had standard debridement procedure additional irrigation by using 50 ml of 10 mg/ml ascorbic acid in normal saline solution during debridement, whereas the control group had debridement only without irrigation with ascorbic acid. The other standard procedure (fixation, rehabilitation, and antibiotic protocols) were done according to the local guidelines.

The culture and colony count of *Staphylococcus aureus* and IL-6 serum level was obtained and measured before and 24 hours after the treatment in both groups. The colony count was defined as the number of *Staphylococcus aureus* colony found in the Chromagar™ *Staphylococcus aureus* plate in 24 hours after culture was initiated. The IL-6 serum level was measured by enzyme-linked immunosorbent assay (ELISA) method. Statistical analysis (Wilcoxon test) was performed by using the SPSS 20 software to compare the difference in the two parameters (Δ colony count and ΔIL-6) before and after the treatment.

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

RESULTS

The study consists of 24 patients as a sample, 18 (75%) male patients and 6 (25%) female patients, with a range of age 17 years to 66 years and a mean age of 32.58 years. 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).

Table 1: Descriptive analysis of open fracture location.

Location	No. of patients	Percentage
Humerus	2	8.3
Antebrachii	2	8.3
Femur	7	29.2
Cruris	13	54.2
Total	24	100

Data of *Staphylococcus aureus* colony in control and treatment group was analyzed descriptively. In the control group, colony of *Staphylococcus aureus* pre and post debridement are 15021.58 CFU/cm^2 and 1507.91 CFU/cm^2 respectively. In the treatment group with ascorbic acid irrigation, colony of *Staphylococcus aureus* pre and post debridement are 1037 CFU/cm^2 and 155.16 CFU/cm^2 respectively (Table 2).

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

Data on the decrease in the number of colonies of *Staphylococcus aureus* germs was obtained by calculating the difference in the number of colonies before and after debridement. Next the data are grouped according to the control and treatment groups and analyzed descriptively. The decrease of *Staphylococcus aureus* colonies in the control and treatment group are 13513.66 CFU/cm^2 and 881.33 CFU/cm^2 respectively (Table 3).

Table 3: Descriptive analysis decreases the number of *Staphylococcus aureus* colonies.

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

The Wilcoxon test results in a decrease in Staphylococcus aureus colonies p=0.308 (p>0.05). Thus a reduction in Staphylococcus aureus colonies was found which was not significant between the control and treatment groups (Table 4).

Table 4: Wilcoxon test decreases *Staphylococcus aureus* colony.

Test statistics[b]	SAvitC - SAcontrol
Z	-1.020[a]
Asymp. Sig. (2-tailed)	0.308

[a]Based on positive ranks, [b]Wilcoxon signed ranks test

Data of IL-6 blood count in control and treatment group was analyzed descriptively. In the control group, IL-6 blood count pre and post debridement are 61.17 pg/ml and 11.37 pg/ml respectively. In the treatment group with ascorbic acid irrigation, IL-6 blood count pre and post debridement are 89.65 pg/ml and 97.63 pg/ml respectively (Table 5).

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

Data on the decrease in the amount of IL-6 blood counts were obtained by calculating the difference in the number of colonies before and after debridement. Next the data are grouped according to the control and treatment groups and analyzed descriptively. The increased IL-6 blood counts in the control and treatment group are 23.97 pg/ml and 7.59 pg/mL respectively (Table 6).

Table 6: Descriptive analysis of IL-6 blood counts.

Group	Colony (CFU/cm^2) mean
Control	23.97
Treatment	7.59

The Wilcoxon test results in a decrease in IL6 levels p=0.239 (p>0.05). Thus, the differences in IL-6 blood counts were found which were not significant between the control group and the treatment group (Table 7).

Table 7: Wilcoxon test decrease IL-6 blood counts.

Test Statistics[b]	IL6 + Vit C - IL6 + control
Z	-1.177[a]
Asymp. Sig. (2-tailed)	0.239

[a]Based on positive ranks, [b]Wilcoxon signed ranks test

DISCUSSION

Analysis of ascorbic acid irrigation post debridement was using *Staphylococcus aureus* colony count because it is the most common microorganism in open fracture. Ascorbic acid irrigation was done to decrease the contamination post debridement and to decrease the risk of infection. Recent study show that ascorbic acid administration in vitro will form 2-4 mm inhibition zone around the *Staphylococcus aureus* agar plate.[10] Other study show that ascorbic acid show that ascorbic acid that given by irrigation in ACL reconstruction can decrease CRP level and promote healing better than normal saline irrigation.[11]

The number of colonies of *Staphylococcus aureus* showed a mean difference in decline, but based on the results of statistical analysis, irrigation of ascorbic acid did not provide additional benefit to reduce the number of contaminant germs 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 (indicated by a significant difference in the number of *Staphylococcus aureus* colonies before treatment) and open fractures in the upper extremities have a lower contamination tendency than the lower extremities. Also, this study does not distinguish the location of open fractures so that variations can occur in terms of the degree of initial contamination and this study does not differentiate the period in hours, so that 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 also only done before the debridement and at the time after treatment, so in this study it was not possible to distinguish at what stage there was a decrease in the number of *Staphylococcus aureus*. Beside those, in this study a dose of 50 ml of 10 mg/ml ascorbic acid was used which has been shown to help accelerate soft tissue healing, but this dose has not been 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 show that ascorbic acid can decreased inflammation significantly by evaluate the hs-CRP level and IL-6 level in diabetic patient.[12] Ascorbic acid administration by irrigation is never been done, so in this study the dosage of ascorbic irrigation based on the research by Fu et al that using irrigation of 50 ml ascorbic

acid 10 mg/ml in ACL reconstruction. In this study ascorbic acid that given by irrigation in ACL reconstruction can decrease CRP level and promote healing better than normal saline irrigation.[11] Ascorbic acid in inflammation process will modulated DNA that bind nuclear factor-kappa B (NF-κB). Inflammation is induced by oxidative stress and will induce cytokine and molecule adhesion in vascular endothelial that will produce tumor necrosis factor- α (TNF-α) and IL-6 by liver. Ascorbic acid can reduce the inflammatory mediator TNF-α and IL-6 by decrease the expression of hepatic mRNA.[12] Ascorbic acid will delay the activation of TNF-α of NF-κB in human in vitro and will decrease the production of GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-3, and IL-5.[14]

The analysis on the effects of treatment administration from the two groups was done by comparing differences in the mean reduction in IL-6 blood counts. The Wilcoxon test results in a decrease in IL6 levels p=0.239 (p>0.05). Thus, the differences in IL-6 blood counts were found which were not significant between the control group and the treatment group. Differences in IL-6 blood counts that are not significant in this study can be caused by: the dose and volume of ascorbic acid solution are inadequate to provide an anti-inflammatory effect because the dosage used is the dose used in arthroscopy for soft tissue healing, and contamination and different degrees of damage to soft tissue cause difference in elevation of IL-6 blood counts, giving rise to 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 there was an increase in IL-6 blood counts and it was not possible to see how the IL-6 blood counts decreased after 24 hours.

CONCLUSION

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

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