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Debridement Using a Combination of 0.9% NaCl Irrigation and Polyhexamethylene Biguanide Reduces C-Reactive Protein, Procalcitonin, and Bacterial Colonization in Patients with Grade II Open Crural Fractures Compared with Debridement Using 0.9% NaCl Irrigation Alone

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ABSTRACT

Background: Grade II open cruris fractures are orthopedic emergencies with a high risk of contamination and infection. Adequate *debridement* and irrigation are crucial to reduce bacterial burden and systemic inflammatory response. Polyhexamethylene biguanide (PHMB) is a broad-spectrum antiseptic that may provide additional benefit compared with normal saline alone.
Objective: To evaluate the effectiveness of combined 0.9% NaCl + PHMB irrigation versus 0.9% NaCl irrigation alone in reducing C-reactive protein (CRP), procalcitonin (PCT), and bacterial colonization in patients with Grade II open cruris fractures. Methods: A clinical trial involving 44 patients with Grade II open cruris fractures at Prof. Dr. IGNG Ngoerah General Hospital, allocated into two groups: 0.9% NaCl (n=22) and 0.9% NaCl + PHMB (n=22). CRP, PCT, and bacterial colonization were assessed pre-operatively and on postoperative day 14. Mann–Whitney tests were used for CRP and PCT, and Chi-square tests for bacterial colonization, with $p<0.05$ considered significant.
Results: Baseline (pre-operative) CRP (p=0.715), PCT (p=0.663), and bacterial colonization (p=0.930) did not differ significantly between groups. On postoperative day 14, the NaCl + PHMB group showed significantly lower CRP levels than the NaCl group (median 2.4 [2.0–3.0] vs 3.2 [2.4–5.0]; $p<0.001$) and significantly lower bacterial colonization ($p<0.001$). PCT levels on day 14 were not significantly different between groups (median 0.09 vs 0.15; p=0.621).
Conclusion: *Debridement* using combined 0.9% NaCl + PHMB irrigation is more effective in reducing CRP and bacterial colonization on postoperative day 14 compared with 0.9% NaCl alone, while no significant difference was observed in PCT levels.

Keywords: open fracture, cruris, *debridement*, irrigation, PHMB, CRP, procalcitonin, bacterial colonization.

INTRODUCTION

Open fractures remain a major orthopedic emergency because disruption of the skin and soft-tissue envelope creates direct communication between bone and the external environment, thereby increasing the risk of contamination, infection, delayed healing, nonunion, and even limb loss if not managed appropriately.[1,2] Lower-extremity open fractures, particularly those involving the tibia and fibula, are among the most frequently encountered long-bone injuries in trauma care.[2,3] The burden of infection rises with increasing severity of soft-tissue injury and contamination, making prompt and adequate early management critical to prognosis.[4,5] In Grade II open crural fractures, the combination of moderate soft-tissue damage and substantial bacterial exposure creates a clinically important setting in which optimization of initial wound treatment may directly influence local wound conditions and systemic inflammatory response.[6]

Debridement and irrigation are central components of open-fracture management.[1] Surgical debridement aims to remove devitalized tissue, foreign material, and contaminants that may serve as a nidus for bacterial proliferation and infection.[6,7] Irrigation complements debridement by reducing bacterial burden, removing debris, and preparing the wound bed for subsequent stabilization and healing.[8] Current treatment principles emphasize early antibiotic administration, meticulous debridement, fracture stabilization, and adequate irrigation volume; however, the optimal irrigation solution remains a subject of ongoing debate.[7] Normal saline is widely used because it is isotonic, readily available, inexpensive, and relatively non-toxic to viable tissue.[8] Nevertheless, saline acts primarily through mechanical cleansing and provides limited direct antimicrobial activity, prompting interest in antiseptic adjuncts that may improve local bacterial control without impairing wound healing.[8,9]

Polyhexamethylene biguanide (PHMB) is a broad-spectrum antimicrobial agent that has gained increasing attention in wound care because of its favorable biocompatibility and activity against Gram-positive bacteria, Gram-negative bacteria, fungi, and biofilm-associated microorganisms.[10,11] PHMB exerts its antimicrobial effect mainly through electrostatic interaction with negatively charged microbial cell membranes, resulting in membrane disruption, increased permeability, and microbial cell death.[11] In addition to its antimicrobial properties, PHMB has been reported to have good tissue tolerability and a low propensity for inducing microbial resistance, making it an attractive candidate for use in contaminated wounds. Previous studies in wound management have suggested that PHMB may reduce infection-related complications and support wound healing, although evidence specific to open long-bone fractures remains limited. [9,12,13]

Early assessment of response after debridement may be enhanced by combining inflammatory and microbiological outcome measures. C-reactive protein (CRP) is a well-established acute-phase reactant synthesized in the liver in response to proinflammatory cytokines, and persistent elevation after trauma or surgery may reflect ongoing inflammation or infection.[14,15] Procalcitonin (PCT), a precursor of calcitonin, has also been used as a biomarker of bacterial infection and systemic inflammatory burden, particularly in more severe infectious states. In parallel, bacterial colonization represents the local microbial load within the wound and may serve as an early surrogate of infection risk before overt clinical infection becomes apparent. [16] Assessment of CRP, PCT, and bacterial colonization together may therefore provide a broader picture of the biological and microbiological effects of different irrigation strategies after debridement [17].

Despite the theoretical advantages of PHMB, comparative clinical evidence between irrigation with 0.9% NaCl alone and irrigation with 0.9% NaCl combined with PHMB in Grade II open crural

fractures is still scarce.[13] This study was therefore conducted to compare debridement using 0.9% NaCl irrigation alone with debridement using a combination of 0.9% NaCl and PHMB irrigation in patients with Grade II open crural fractures. The primary objective was to evaluate differences in postoperative CRP, procalcitonin, and bacterial colonization between the two approaches. We hypothesized that the addition of PHMB to standard saline irrigation would provide greater reduction in inflammatory biomarkers and bacterial colonization than saline irrigation alone.

MATERIALS & METHODS

Study Design and Setting

This study was a single-center clinical trial comparing two irrigation strategies used during initial debridement in patients with Grade II open crural fractures. The study was conducted at RSUP Prof. Dr. I.G.N.G. Ngoerah, Denpasar, Bali, with patient recruitment from the emergency department and follow-up assessments performed through the orthopedic outpatient service, clinical pathology laboratory, and microbiology laboratory. Data collection was completed over a 3-month study period.

Participants

The study population consisted of adult patients presenting with Gustilo-Anderson Grade II open crural fractures who had received intravenous ceftriaxone prior to surgery. Eligible patients were recruited from the emergency department and were included after providing informed consent. Inclusion criteria were adult age, either sex, confirmed Grade II open crural fracture, prior administration of intravenous ceftriaxone, and performance of initial debridement using either 0.9% NaCl alone or 0.9% NaCl combined with polyhexamethylene biguanide (PHMB). Patients were excluded if they had major comorbid conditions that could influence wound healing or inflammatory response, including diabetes mellitus, malignancy, or autoimmune disease, or if they declined participation. Patients were considered dropouts if they withdrew before completion of follow-up or died during treatment before outcome assessment.

Sample size was calculated using the formula for comparison of two independent numerical means based on the expected difference in CRP values reported in previous literature. The minimum sample size was 20 patients per group; after allowing for 10% loss to follow-up, the final required sample size was 22 patients in each group, for a total of 44 patients. In the final analysis, 44 patients were allocated equally into two groups: 22 patients underwent debridement with 0.9% NaCl irrigation alone and 22 underwent debridement with 0.9% NaCl plus PHMB irrigation.

Intervention and Comparator

All patients underwent initial surgical debridement under general anesthesia following standard antiseptic and aseptic preparation. In the comparator group, the wound was irrigated repeatedly with 6 L of 0.9% NaCl until gross contamination had been removed. In the intervention group, the wound was first irrigated with PHMB solution (Killbac) and was subsequently irrigated with 6 L of 0.9% NaCl. In both groups, the wound was enlarged when necessary to allow adequate exposure, nonviable or avascular wound edges were excised, and wound closure was performed according to the condition of the wound; wounds considered sufficiently clean were sutured, whereas contaminated wounds were left open.

Outcome Measures

The primary outcomes were C-reactive protein (CRP), procalcitonin (PCT), and bacterial colonization. CRP and PCT were assessed preoperatively and again on postoperative day 14 using venous blood samples processed in the hospital clinical pathology laboratory. Both variables were analyzed as numerical outcomes.

Bacterial colonization was evaluated using wound swab specimens obtained before debridement and again on postoperative day 14. After superficial debris or exudate had been removed with sterile normal saline, samples were collected using a sterile swab with the Levine technique by rotating the swab over a 1 cm^2 area of clean granulation tissue with sufficient pressure to express wound fluid. Swabs were placed in transport medium and sent to the microbiology laboratory within 2 hours. Samples were cultured on agar media using a four-quadrant streak method, incubated at 37°C for 18-24 hours, and interpreted by a clinical microbiologist. Colonization was reported as a semi-quantitative ordinal variable: +1, very light growth limited to the first quadrant; +2, light growth up to the second quadrant; +3, moderate growth up to the third quadrant; and +4, heavy growth extending to the fourth quadrant or across the full culture surface.

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS). Descriptive data were presented as mean ± standard deviation for normally distributed numerical variables or as median with interquartile range or range for non-normally distributed variables, while categorical variables were presented as frequency and percentage. Normality was assessed using the Shapiro-Wilk test because each study arm contained fewer than 50 participants. Based on the final distribution of the data, between-group comparisons for CRP and PCT were analyzed using the Mann-Whitney U test, and bacterial colonization was compared using the chi-square test or Fisher's exact test when appropriate. A two-sided p value of less than 0.05 was considered statistically significant.

RESULT

A total of 44 patients with Grade II open crural fractures were included in the study and were equally allocated into the 0.9% NaCl group (n=22) and the 0.9% NaCl + PHMB group (n=22). In the NaCl 0.9% group, 10 patients (45.5%) were male and 12 (54.5%) were female, whereas in the NaCl 0.9% + PHMB group, 14 patients (63.6%) were male and 8 (36.4%) were female. The mean age was 49.2 ± 13.5 years in the NaCl 0.9% group and 49.0 ± 12.3 years in the NaCl 0.9% + PHMB group, indicating generally comparable baseline characteristics between groups (Table 1).

Table 1. Baseline characteristics of the study participants

Variable	NaCl 0.9% (n=22)	NaCl 0.9% + PHMB (n=22)
Male, n (%)	10 (45.5)	14 (63.6)
Female, n (%)	12 (54.5)	8 (36.4)
Age, mean ± SD	49.2 ± 13.5	49 ± 12.3

Preoperative CRP levels did not differ significantly between the two groups. The median preoperative CRP was 3.6 mg/L (2.5-5.5) in the NaCl 0.9% group and 3.7 mg/L (2.4-5.8) in the NaCl 0.9% + PHMB group (p=0.715). On postoperative day 14, CRP levels were significantly lower in the NaCl 0.9% + PHMB group than in the NaCl 0.9% group, with median values of 2.4 mg/L (2-3) and 3.2 mg/L (2.4-5), respectively (p<0.001) (Table 2).

Table 2. Comparison of CRP levels between groups

Time Point	NaCl 0.9%	NaCl 0.9% + PHMB	p-value
Preoperative	3.6 (2.5-5.5)	3.7 (2.4-5.8)	0.715
Postoperative day 14	3.2 (2.4-5)	2.4 (2-3)	<0.001

Data are presented as median (min-max).

Preoperative procalcitonin levels were also comparable between groups. The median preoperative PCT was 0.68 ng/mL (0.15-1.05) in the NaCl 0.9% group and 0.69 ng/mL (0.13-1.05) in the NaCl 0.9% + PHMB group (p=0.663). At postoperative day 14, the median PCT was 0.15 ng/mL (0.03-0.25) in the NaCl 0.9% group and 0.09 ng/mL (0.03-0.25) in the NaCl 0.9% + PHMB group; however, this difference was not statistically significant (p=0.621) (Table 3).

Table 3. Comparison of procalcitonin levels between groups

Time Point	NaCl 0.9%	NaCl 0.9% + PHMB	p-value
Preoperative	0.68 (0.15-1.05)	0.69 (0.13-1.05)	0.663
Postoperative day 14	0.15 (0.03-0.25)	0.09 (0.03-0.25)	0.621

Data are presented as median (min-max).

Bacterial colonization at baseline was similar between groups (p=0.930). In the preoperative assessment, the NaCl 0.9% group showed colonization grades of +1 in 1 patient (4.5%), +2 in 9 patients (40.9%), +3 in 9 patients (40.9%), and +4 in 3 patients (13.6%). The NaCl 0.9% + PHMB group showed +1 in 1 patient (4.5%), +2 in 7 patients (31.8%), +3 in 11 patients (50.0%), and +4 in 3 patients (13.6%). On postoperative day 14, bacterial colonization differed significantly between groups (p<0.001). The NaCl 0.9% group remained dominated by higher colonization grades, with +1 in 1 patient (4.5%), +2 in 7 patients (31.8%), +3 in 11 patients (50.0%), and +4 in 3 patients (13.6%). In contrast, the NaCl 0.9% + PHMB group showed a shift toward lower colonization grades, with +1 in 10 patients (45.5%), +2 in 9 patients (40.9%), +3 in 3 patients (13.6%), and no patients with +4 colonization (Table 4).

Table 4. Comparison of bacterial colonization between groups

Time Point	Group	+1	+2	+3	+4	p- value
Preoperative	NaCl 0.9%	1 (4.5)	9 (40.9)	9 (40.9)	3 (13.6)	0.930
	NaCl 0.9% + PHMB	1 (4.5)	7 (31.8)	11 (50)	3 (13.6)	
Postoperative day 14	NaCl 0.9%	1 (4.5)	7 (31.8)	11 (50)	3 (13.6)	<0.001
	NaCl 0.9% + PHMB	10 (45.5)	9 (40.9)	3 (13.6)	0 (0)	

Data are presented as n (%).

Overall, the addition of PHMB to 0.9% NaCl irrigation was associated with significantly lower CRP levels and lower bacterial colonization on postoperative day 14, while no significant between-group difference was observed for procalcitonin.

DISCUSSION

Baseline Characteristics

This study included 44 patients with Grade II open crural fractures, equally allocated into the 0.9% NaCl group and the 0.9% NaCl + PHMB group. Baseline characteristics were generally comparable between groups, particularly with respect to age, with mean ages of 49.2 ± 13.5 years in the NaCl 0.9% group and 49.0 ± 12.3 years in the NaCl 0.9% + PHMB group. Although the sex distribution was not identical, both groups were similar enough to support comparison of postoperative outcomes. Assessment of baseline comparability is important in comparative clinical studies because it helps reduce the likelihood that differences in outcomes are explained by pre-existing group imbalance rather than by the intervention itself.[18] Epidemiologically, open tibial fractures are more common in males and are frequently associated with trauma in active adult populations, so the demographic distribution observed in this study remains clinically plausible.[19]

C-Reactive Protein (CRP)

The present study showed no significant between-group difference in preoperative

CRP levels, suggesting that the initial inflammatory status was broadly similar before intervention. However, by postoperative day 14, CRP was significantly lower in the NaCl 0.9% + PHMB group than in the NaCl 0.9% group. This finding may be clinically relevant when interpreted in the context of CRP kinetics after trauma and surgery. Previous reports have shown that CRP typically rises rapidly after tissue injury, peaks within the early postoperative period, and then gradually declines toward baseline over the following one to two weeks in uncomplicated cases. [20] Persistently higher CRP values during this later phase may therefore reflect sustained inflammatory stimulation, including possible ongoing bacterial burden. In this context, the lower day-14 CRP level in the PHMB group may indicate more rapid resolution of local inflammatory activity.[21]

A possible biological explanation for this finding is the difference in wound bioburden control between the two irrigation strategies. Normal saline primarily provides mechanical cleansing, whereas PHMB offers additional broad-spectrum antiseptic activity and has been reported to have good safety and tolerability in wound applications. [22,9] Experimental and clinical studies on polihexanide-based wound care have shown reduction in bacterial load and support the plausibility that improved local antimicrobial control may attenuate postoperative inflammatory response.[22] Nevertheless, CRP is a nonspecific acute-phase reactant and may also be influenced by injury severity, soft-tissue damage, timing of debridement, and other perioperative variables. [21,23] Therefore, the present findings are best interpreted as showing an association between PHMB use and lower CRP at postoperative day 14 rather than definitive proof of reduced infection.

Procalcitonin (PCT)

In contrast to CRP, procalcitonin did not differ significantly between groups either preoperatively or on postoperative day 14, although the PHMB group showed a numerically lower median value at follow-up. This result is consistent with the known postoperative kinetics of procalcitonin. In surgical and traumatic settings, PCT tends to increase earlier than CRP, often peaking during the first postoperative day, and then declines relatively quickly when systemic infection is absent or adequately controlled.[24] Because the present study measured PCT only at baseline and day 14, any difference occurring during the early postoperative peak may not have been captured. This timing issue may partly explain why no statistically significant difference was observed at the later follow-up point. [20]

Another consideration is that PCT is generally more closely associated with systemic bacterial infection than with localized wound contamination or early local inflammatory changes. In orthopedic settings, PCT may remain normal or normalize quickly even when localized infection or wound-related bacterial processes are still evolving. [25]Accordingly, the absence of a significant between-group difference in PCT does not necessarily contradict the observed CRP and colonization findings. Rather, it suggests that PCT may have been less sensitive than CRP for distinguishing the biological impact of the two irrigation strategies at postoperative day 14. In this study, PCT may therefore be better viewed as a complementary biomarker rather than the primary marker of early local wound response.[8]

Bacterial Colonization

Bacterial colonization findings provide the strongest direct microbiological support for the use of PHMB in this study. Colonization grades were comparable between groups preoperatively, but by postoperative day 14 the NaCl 0.9% + PHMB group showed a clear shift toward lower colonization grades, with more patients in the +1 and +2 categories and no patients in the +4 category. By contrast, the NaCl 0.9% group

remained dominated by higher colonization grades. This pattern supports the hypothesis that adding PHMB to saline irrigation may improve local bacterial control after debridement in Grade II open crural fractures.[26]

This finding is consistent with the general principle that irrigation and debridement in open fractures are intended to reduce bacterial burden, remove contaminants, and preserve viable tissue in order to lower infection risk. [27] Normal saline remains the standard irrigation solution in many settings because of its availability and tissue compatibility, but it acts mainly through physical lavage. PHMB, on the other hand, has broad antimicrobial activity and has also been associated with anti-biofilm effects, which may be especially relevant in contaminated traumatic wounds where persistent bacterial adherence can contribute to later wound complications. The direction of the present findings is also in line with previous wound-care literature suggesting that polyhexanide-containing solutions can reduce bacterial burden more effectively than simple irrigation alone. [28]

Even so, these results should be interpreted with caution. Bacterial colonization is a surrogate microbiological outcome and is not equivalent to clinically confirmed surgical site infection or fracture-related infection. Colonization may also be influenced by the extent of initial contamination, time to surgery, antibiotic exposure, wound condition, and the semi-quantitative nature of the culture grading system used in this study. In addition, the study had a relatively small sample size and follow-up was limited to 14 days, which restricts conclusions regarding longer-term outcomes such as osteomyelitis, re-debridement, nonunion, or reoperation. Overall, however, the combined pattern of lower CRP and lower bacterial colonization at postoperative day 14 suggests that PHMB may provide an early adjunctive benefit when added to 0.9% NaCl irrigation during debridement of Grade II open crural fractures. [26]

CONCLUSION

In patients with Grade II open crural fractures, debridement using a combination of 0.9% NaCl and polyhexamethylene biguanide (PHMB) irrigation was associated with lower postoperative C-reactive protein levels and reduced bacterial colonization at postoperative day 14 compared with debridement using 0.9% NaCl irrigation alone, while no significant difference was observed in procalcitonin levels. These findings suggest that adding PHMB to standard saline irrigation may provide an early benefit in controlling local bacterial burden and promoting a more favorable inflammatory response after debridement. However, given the relatively small sample size, short follow-up duration, and the use of semi-quantitative colonization assessment, further larger prospective studies with longer follow-up and clinically definitive outcomes are needed to confirm its effect on infection prevention and overall fracture-related outcomes.

Declaration by Authors

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