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Differences in Decreasing Bacteria Colonization Wound Irrigation using 0.9% Nacl Compared with Wound Irrigation using Active Ingredients Polyhexanide 0.1% and 0.1% Betaine in Open Fractures of Long Bone

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Abstract

BACKGROUND: An open fracture is an emergency case that must be treated quickly to prevent infection. The use of an irrigation solution containing 0.1% of the antimicrobial polyhexanide and 0.1% of the surfactant betaine has been associated with more satisfactory wound healing.

AIM: This study was comparing irrigation with polyhexanide 0.1% and betaine 0.1% (P + B) in reducing bacterial colonization of open fractures compared to using 0.9% natrium chloride (NaCl) solution.

METHODS: This study uses a pre- and post-control group design. Sampling was carried out in the operation room of the emergency care installation, inpatient installation, and outpatient installation of prof. Hospital. Dr. I.G.N.G Ngoerah Denpasar, between September and November 2022. From a sample population suffering from open fractures of the lower extremities, samples of patients were taken who were irrigated with 0.9% NaCl solution with a mixture of polyhexanide 0.1% and betaine 0.1%. The presence or absence of bacterial colonization was examined using a surgical wound base swab with the Levine technique.

RESULTS: In the 30 patients in this study, there was a significant decrease in bacterial levels (Log colony-forming unit [CFU]/g) after irrigation, both from irrigation with NaCl ($p = 0.04$) and NaCl + P + B ($p < 0.001$). The reduction in bacterial levels was more significant with NaCl + P + B compared with NaCl alone, at post-irrigation ($p < 0.001$), H+3 ($p = 0.003$), and H + 7 ($p < 0.001$).

CONCLUSION: Wound irrigation using a combination of 0.9% NaCl, 0.1% polyhexanide, and 0.1% betaine reduced bacterial colonization more than using 0.9% NaCl-only solution in open fractures of long bones.

Introduction

Open fractures are fractures through which the skin and surrounding soft tissue are damaged so that the bone fragments come into contact with the outside environment. This open fracture is an emergency case in the field of orthopedics which must be treated quickly to prevent infection or failure to heal from the bone itself. Infection is one of the main causes of impaired bone healing process and poor outcome in patients with open fractures, especially long bone fractures. Treatment of open fractures in long bone such as debridement and irrigation is very important because it is also one of the factors that can determine the final outcome of this case. At present, there are several materials that are widely used in the irrigation process, namely, using 0.9% natrium chloride (NaCl) solution or mixtures with active ingredients such as active polyhexanide 0.1% and 0.1% betaine.

The incidence of open fractures is much higher in developing countries. Globally, in 2019, there were 178 million fracture cases (33.4% increase since 1990). The incidence rate of open fractures reached 30 open fractures per 100,000 people each year which are usually caused by severe injuries, especially motor vehicle accidents, which is most prevalent in the young and productive age group [1]. The incidence of fractures in Indonesia was recorded at 5.5%. Meanwhile, the incidence of fractures in Bali reaches a prevalence of up to 7.5% [2]. Meanwhile, for the prevalence of injuries by body part, injuries to the lower extremities had the highest prevalence, namely, 67.9% [3]. In cases of open fractures, the prevalence of infection rates at each degree is also different. The higher the degree of an open fracture, the higher the risk of infection [4], [5]. According to Gustilo, the degree of infection is directly correlated with the type of fracture. In grade 1 open fractures, the risk of infection is 1.9%, in grade 2, it is 8%, while, in fractures, it increases to 41% [6].

The basic principles of initial treatment of open fractures include initial immobilization, tetanus prophylaxis, administration of antibiotics, irrigation, and debridement. Thorough debridement and irrigation are key pillars of successful management of open fractures. The last-mentioned action plays a very important role because it is expected to reduce the number of microorganism colonies to prevent direct infection. Apart from the amount, the type of fluid used for wound irrigation is equally important. There are various types of fluids that can be used, including physiological saline, sterile water, tap water, antibiotic solutions, castile soap, benzalkonium chloride, chlorhexidine, hydrogen peroxide (H_2O_2), and povidone iodine (PI), each of which has its advantages and disadvantages. Physiological saline or 0.9% NaCl is the standard fluid commonly used to irrigate wounds. 0.9% NaCl is isotonic and non-toxic to normal healing processes and is relatively inexpensive [7]. Then, there is another irrigation fluid in the form of a mixture of 0.1% polyhexanide and 0.1% betaine which can work on biofilms formed by bacteria [7], [8]. However, there are major concerns related to the use of other irrigation fluids due to side effects or effectiveness in reducing the number of bacterial colonies which are considered less than optimal. For example, H_2O_2 as an irrigation fluid has the effectiveness of reducing the number of bacterial colonies but have cytotoxicity and the potential for air embolism. The same thing applies to PI solutions which are known to have toxicity that is not related to the concentration of the solution but is more related to an exposure time [9].

Based on the description above and taking into account how important wound irrigation and debridement are in cases of open fractures, it is necessary to conduct further research regarding the effectiveness of the type of fluid used for irrigation. Therefore, this study aims to evaluate the effectiveness of irrigation with fluids with active ingredients of 0.1% polyhexanide and 0.1% betaine in reducing bacterial colonization of open fractures in long bone compared to using 0.9% NaCl solution.

Methods

This study uses a experimental pre- and post-control group design. From the sample population suffering from open fractures of the lower extremities, samples of patients were taken who were irrigated with 0.9% NaCl solution with a mixture of polyhexanide 0.1% and betaine 0.1%. The presence or absence of bacterial colonization was examined using a surgical wound base swab using the Amies Swab with the Levine technique 4 times, namely, before standard debridement and irrigation; after standard debridement and irrigation; 3 days after standard debridement and irrigation; 7 days post-standard debridement and irrigation to see if there is a difference in the number of bacterial colonies from samples taken from the wound. Sampling was carried out in the operation room of the Emergency Care Installation, Ward Installation, and Outpatient Installation of Prof. Dr. I.G.N.G Ngoerah Hospital, during the September–November 2022 period.

This study uses consecutive sampling. Afterward, all samples were randomized using an online randomizer application. In this study, the sample size was calculated using the Pocock formula, and a minimum sample of 15 people was obtained including an anticipated drop-off of 20% [10].

Colony-forming unit (CFU) is a unit of measurement of a population of cells that can be seen with the naked eye in a growth medium. In its measurement, the culture media is divided into four quadrants, if in the first quadrant, there is bacterial growth that it is considered with a value of 10^2, the second quadrant is 10^3, the third quadrant is 10^4, and the fourth quadrant is 10^5. Growth in the last quadrant is the benchmark for the number of microorganism growth. The patient was then given broad-spectrum antibiotics and anti-tetanus according to the applicable standard procedure at Prof. Dr. I. G.N.G Ngoerah Hospital (antibiotics with ceftriaxone two grams IV pre-operative and continued with 2 × 1 g IV). The patient then underwent a standard debridement operation followed by two different irrigation methods, namely, using irrigation only with 0.9% NaCl or with 0.9% NaCl plus a mixture of active compounds polyhexanide 0.1% and betaine 0.1% 350 mL without diluting.

Results

In this study, a sample of 30 patients was obtained. Table 1 has mentioned the mean age of the patients was 42 years. There were 20 male patients, while ten female patients each had an average age of 32 years and 48 years, respectively.

The research variables were tested for normality using the Shapiro–Wilk test, which is more suitable for a sample size of <50. Table 2 shows that the post-irrigation NaCl group, H + 3 NaCl irrigation, and H + 7 0.9% NaCl irrigation did not have a normal distribution.

Table 1: Distribution of the characteristics of the research subjects

Variable	Number (%)	Mean ± SD
Age		42 ± 13
Gender		
Male	20 (66.6)	
Female	10 (33.3)	

Table 2: Data normality test

Group	n	p-value	Distribution
NaCl pre-irrigation	15	p > 0.05	Normal distribution
NaCl + P + B pre-irrigation	15	p > 0.05	Normal distribution
NaCl post-irrigation	15	p = 0.004	Not normally distributed
NaCl + P + B post-irrigation	15	p > 0.05	Normal distribution
H + 3 NaCl irrigation	15	p = 0.002	Not normally distributed
H + 3 NaCl + P + B irrigation	15	p > 0.05	Normal distribution
H + 7 NaCl irrigation	15	p = 0.005	Not normally distributed
H + 7 NaCl + P + B irrigation	15	p > 0.05	Normal distribution

NaCl: Natrium chloride 0.9%; NaCl + P + B: Natrium chloride 0.9% + Polyhexanide 0.1% + Betaine 0.1%.

Table 3 shows the results of 0.9% NaCl irrigation treatment and polyhexanide 0.1% + Betaine 0.1% at different sampling times, which are then cultured with MacConkey agar or blood agar. Bacterial load expressed in CFU units CFU per gram which is carried out by logarithmic transformation to facilitate the calculation of exponential CFU into Log CFU groups. The dependent t-test was carried out to see the significance of the decrease in post-irrigation NaCl + P + B, H + 3 NaCl + P + B irrigation, and H + 7 NaCl + P + B irrigation groups. Wilcoxon signed-rank test was carried out to see the significance of the decrease in the groups without a normal distribution, which was NaCl post-irrigation, H + 3 NaCl irrigation, and H + 7 NaCl irrigation groups. There was a significant decrease in Log CFU/g after irrigation, both from irrigation with NaCl (p = 0.04) as well as NaCl + P + B (p < 0.001). The results are shown in Figure 1.

Table 3: Decrease in Log CFU/g between the treatment of the 0.9% NaCl group and the 0.9% NaCl + Polyhexanide 0.1% + Betaine 0.1% group on pre-irrigation, post-irrigation, H + 3 irrigation, and H + 7 irrigation

Group	NaCl (Log CFU/g)	p-value	NaCl + P + B (Log CFU/g)	p-value
Pre	5.40 ± 0.22	-	5.41 ± 0.20	-
Post	5.29 ± 0.25	p = 0.04[b]	4.68 ± 0.29	p < 0.001[a]
H + 3	4.34 ± 0.23	p = 0.001[b]	3.94 ± 0.39	p < 0.001[a]
H + 7	4.12 ± 0.27	p = 0.001[b]	3.71 ± 0.36	p < 0.001[a]

[a]Dependent t-test, [b]Wilcoxon signed-rank test, CFU: Colony-forming unit, NaCl: Natrium chloride 0.9%, NaCl + P + B: Natrium chloride 0.9% + Polyhexanide 0.1% + Betaine 0.1%.

Table 4 shows the results of 0.9% NaCl irrigation, polyhexanide 0.1%, and betaine 0.1% at different sampling times. Kruskal–Wallis's test was carried out for the post-irrigation, H + 3, and H + 7 groups with 0.9% NaCl irrigation. No significant differences were found in the bacterial colonies between the two groups before irrigation (p = 0.85). There was a significant difference

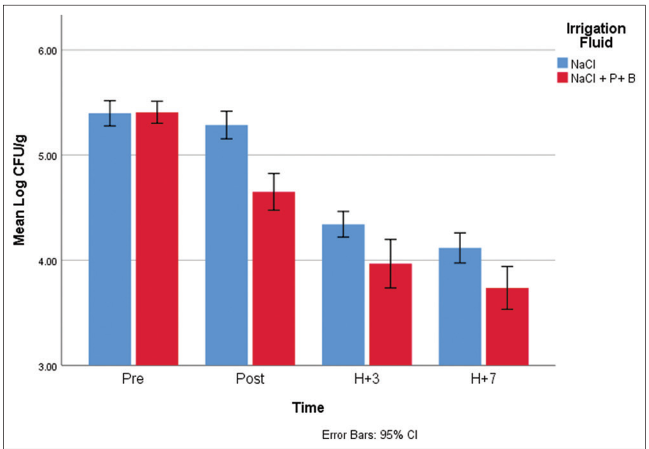

Figure 1: Visualization of the decrease in bacterial colonies (colony-forming unit/g) between groups of irrigation fluidse

in the reduction of bacterial colonies between groups after irrigation (p < 0.001), H+3 irrigation (p = 0.003), and H + 7 irrigation (p < 0.001).

Table 4: Differences in Log CFU/g reduction between the 0.9% NaCl and 0.9% NaCl + Polyhexanide 0.1% + Betaine 0.1% group on pre-irrigation, post-irrigation, H + 3 irrigation, and H + 7 irrigation

Group	Log CFU/g	Log CFU/g Reduction	Differences in Log CFU/g Reduction (95% CI)	p-value
NaCl pre-irrigation	5.40 ± 0.22	-	-	p = 0.85[a]
Pre-irrigation NaCl + P + B	5.41 ± 0.20	-		
Post-irrigation NaCl	5.29 ± 0.25	0.21 ± 0.30	0.56 (0.33–0.79)	p < 0.001[b]
Post-irrigation NaCl + P + B	4.68 ± 0.29	0.77 ± 0.34		
H + 3 NaCl irrigation	4.34 ± 0.23	1.07 ± 0.24	0.48 (0.24–0.73)	p = 0.003[b]
H + 3 NaCl + P + B irrigation	3.94 ± 0.39	1.55 ± 0.42		
H + 7 NaCl irrigation	4.12 ± 0.27	1.32 ± 0.33	0.40 (0.11–0.69)	p < 0.001[b]
H + 7 NaCl + P + B irrigation	3.71 ± 0.36	1.72 ± 0.47		

[a]Independent t-test, [b]Kruskal–Wallis test. CFU: Colony-forming unit, NaCl: Natrium chloride 0.9%, NaCl + P + B: Natrium chloride 0.9% + Polyhexanide 0.1% + Betaine 0.1%.

Discussion

Wound healing is a complex process involving continuous biocellular and biochemical activities. The combination of vascular response, cellular activity, and the formation of chemicals as mediator substances in the wound area are interrelated components in the wound healing process. Wound healing varies depending on the location, severity, and extent of the injury. The ability of cells and tissues to regenerate or return to normal structure through cell growth also affects wound healing.

Sodium chloride is a physiological solution that exists throughout the body, and thus, this antiseptic does not elicit a hypersensitivity reaction upon application. Based on the results obtained in this study, irrigation in cases with open fractures of long bones using 0.9% NaCl solution was able to reduce bacterial colonization in several periods of observations. The same results were also found in irrigation using active ingredients polyhexanide 0.1% and betaine 0.1%. This shows that these two materials can reduce bacterial colonization in long bone open fractures. When the two groups were compared, irrigation using polyhexanide 0.1% and betaine 0.1% reduced bacterial colonization more than irrigation using only 0.9% NaCl in open fractures of long bones.

Polyhexanide has been shown as an antimicrobial polymer that is effective against intracellular forms and biofilms of *Staphylococcus aureus* [11]. Polyhexanide as a strong base has bactericidal properties, binds to negatively charged phosphates on the phospholipid membrane of bacteria, destroys the outer and inner membranes of the bacterial cell wall through permeability disturbances, and excretes the cytoplasm of bacterial cells by osmosis [12]. Brill *et al*., shows that mechanical rinsing with polyhexanide 0.02% solution was significantly and consistently more effective in reducing bacterial colonization than 0.9% NaCl solution [13].

There are major concerns related to the use of other irrigation fluids mainly due to side effects. H_2O_2 is often used in wound irrigation, but at the time of use and there is a contact reaction with catalase in the tissue that will experience degradation into water and oxygen. This reaction will elicit microbubbles, which can cause fatal pulmonary embolism [14]. Yang *et al*., also argued that the solution of H_2O_2 cannot be used as an antiseptic, since the antibacterial ability will disappear faster than the abovementioned toxicity [15].

H_2O_2 also adversely affects the articular cartilage by inhibiting the normal metabolic functions of chondrocytes. This is due to depletion of adenosine triphosphate in cells, and reduced synthesis of cartilage proteoglycans and hyaluronic acid [16]. In addition, the reaction can trigger the desquamation of H_2O_2 into the water and oxygen produced by the reactions can enter the circulation and trigger the release of histamine and bradykinin, which will lead to hypersensitivity reactions and even shock [17].

Povidone Iodine is known to have toxicity that is not related to the concentration of the solution but rather related to an exposure time. Regarding the combination of the two solutions, the solution (0.3% PI + H_2O_2 0.5%) showed more significant toxicity after 1 min of exposure time. Higher levels of toxicity have been shown for H_2O_2 with a concentration of 1.5% at a long exposure of 5 min [9]. Therefore, this study uses polyhexanide as a safer and effective irrigation fluid.

This study does not consider the effects of varying volume and concentration of the polyhexanide and betaine solution used on bacterial load reduction and side effects to tissues. In addition, the bacterial load reduction on day 3 and day 7 could be obfuscated by the effects of antibiotics.

Conclusion

This study showed that irrigation using 0.9% NaCl solution can significantly reduce bacterial colonization in cases of open fractures of long bones. Addition of polyhexanide 0.1% and betaine 0.1% to the irrigation fluid can further increase the effectiveness of bacterial load reduction in this condition. Further, study is needed in analyzing the volume and concentration of polyhexanide and betaine combinations in patients with open fractures.

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