



Dilution of Open Fracture Grade IIIA of the Lower Leg Using Normal Saline 0.9% Mixed with Honey Compared With Normal Saline 0.9% to the Bacterial Growth



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Abstract

Patients who experienced grade IIIA open fracture of the lower leg tend to suffer from a high incidence of infections even after management of wound irrigation, antibiotic administration, and surgical debridement in operating room. This study aims to focus on a way to reduce bacterial colony, dilute bacterial concentration, and remove foreign bodies in the wound so that the bacterial number can be minimized.

Honey has been used for centuries as a dressing medium for infected wound care. Honey has an anti-bacterial activity in the inhibitory factors which act as peroxide hydrogen so that it may decrease the incidence of infection and theoretically can be used in patients with open fracture grade IIIA. Peroxide hydrogen is produced by honey after reaction with the glucose oxidase enzyme which becomes active when the honey is diluted.

This is an experimental study with 28 patients' samples divided into two groups, underwent wound irrigation: [1] with honey-mixed 0.9% normal saline and [2] with 0.9% normal saline only. There was no significant difference in both groups' pre-treatment data of bacterial count. The treatment is then evaluated by comparing bacterial count before and after treatment. In the first group, bacterial count is found to be smaller in 12 patients' sample after dilution with 0.9% NaCl and honey compared with the count before dilution (negative difference) and has the rank number (R1) of 78.00, while 2 samples showing the same number of bacteria colonies after and before dilution. No sample shows a greater number of bacteria colonies after dilution (positive difference).

In the other group, bacterial count is found to be smaller [3] in 13 patients' sample after dilution with 0.9% NaCl (without honey mixture) compared with the count before dilution (negative difference) and has the rank number (R1) of 95.00, but a greater number of bacteria colonies after dilution (positive difference) is found in 1 patient sample and has the rank number (R2) of 10.00[4].

There is significant difference of post-treatment data in the two groups. The bacterial count in samples treated with honey-mixture 0.9% NaCl were significantly lowered compared to the bacterial count in samples treated with 0.9% NaCl only [5]. This study concluded that open fracture grade IIIA wound dilution with honey-mixture 0.9% NaCl is superior than wound dilution with 0.9% NaCl only.

Keywords: Open fracture; Honey; Normal saline

Introduction

The incidence of traffic accidents in Indonesia has increased from 2007 (48,508 cases) to 2008 (59,164 cases) and in 2009 (62,960 cases). One of many results of these traffic accidents is fracture of the long bone [6]. These fractures are at risk for an impaired healing due to any connection with external environment. Contamination incidence of open fracture wounds in Dr. Hasan Sadikin hospital in 1994 is 55.1% and increased 91% in 1998.

One of the important management of handling patients with open fractures is cleansing the wound, besides administration

of appropriate antibiotic according to the bacterial culture, and immobilizing fracture segments with external fixation [7]. It is one of the keys to prevent any infection in open fractures because it dilutes the concentration of bacteria, removing foreign bodies in the wound and reduces the number of bacterial colonies.

Various ways have been attempted to reduce the number of colonies of germs in patients with lower extremity wounds open fractures grade III A, by cleansing the wound with 0.9% NaCl, washing wounds using boiled water, using a mixture with povidone

iodine, but the infection rate is still high in patients with open fractures of lower leg grade III A, therefore the author have an idea of using a mixture of 0.9% NaCl and honey [8].

The purpose of cleansing the wound with 0.9% NaCl is to dilute the concentration of bacteria, remove foreign bodies in the wound, so that when debridement is done, the number of colonies of bacteria and germs has been reduced [9]. Honey has an anti-bacterial activity and inhibine factors which can decrease the incidence of infection so that honey can be used in patients with open fractures grade IIIA. Hydrogen peroxide is produced by honey after reaction with the enzyme glucose oxidase which becomes active when the honey is diluted. The use of honey as a mixture with NaCl 0.9% in irrigating fracture lower leg injuries grade IIIA will help lower the number of colonies of germs that will reduce the incidence of infection. The purpose of this study was to evaluate the use of a mixture of NaCl 0.9% - honey in wound irrigation in open fractures of lower leg grade IIIA in the ER, whether it is the same, better or worse in reducing the number of bacteria colonies compared to using 0.9% NaCl [10].

Method

This research is an experimental study by comparing the wound diluted using 0.9% NaCl and honey mixture and 0.9% NaCl alone. These research subjects have to meet the inclusion and exclusion criteria. Inclusion criteria included: age 16-55 years, patients injured lower limb fractures open grade IIIA caused by traffic accidents who have not received medical procedures such as irrigation and administration of prophylactic antibiotics since the accident, which is less than or equal to 6 hours [11]. The exclusion criteria is when the subject is under treatment of antibiotics or corticosteroids.

The procedure of the study include informed consent and filling the consent form, identity record, wound irrigation by using a mixture of 0.9% NaCl and honey for the first group and irrigation of the wound with NaCl 0.9% only for the second group, and last, sample taking with simple random sampling.

Wound cleansing on the subjects of the first group use a mixture of 0.9% NaCl and honey, prepared by injecting 100 grams of honey (weighed with digital scales) by using a 50cc sterile syringe container into 500mL kolf of NaCl 0.9% at Hasan Sadikin Hospital emergency department. Wound cleansing on the subjects in the second group use plain 0.9% NaCl. Irrigation technique is based on pressure irrigation system for open fracture wounds that

Table 2: The difference between Bacterial colony before and after irrigating the wound of Open Fracture of lower leg grade IIIA with mixture of NaCl 0.9 % and Honey.

	N	Mean Rank	Sum of Ranks
NaCl 0.9% - honey (PostTest) - NaCl 0.9% - honey (PreTest)	Negative Ranks	12	78,00
	Positive Ranks	0	0,00
	Ties	2	
Total	14		

uses as much as 9 liters of fluid conducted in the ER by pressing the packaging of 0.9% NaCl until it was emptied [12].

A sample was taken from the base of the wound before and after dilution by using a sterile cotton swab stick at the bottom of the wound from both groups of research subjects and was put into a sterile test tube by author assisted by a trained assistant then taken to the laboratory of Clinical Pathology Department of Clinical Microbiology Division Hasan Sadikin General Hospital.

The sample was taken from the test tube by using a loop stick size of 10 microns, then the sample is grown / cultured in a petri dishes with Mueller-Hinton media and kept in an incubator at 37 °C for 24 hours [13]. After 24 hours, the growing bacteria is counted.

The bacteria count is carried on the 2nd day directly by counting the number of colonies one by one based on the growing bacteria on the petri dishes with Mueller-Hinton media [14]. Furthermore, the data acquired is analyzed with non-parametric statistical tests Wilcoxon signed-ranks for the paired data and Mann-Whitney test for independent data in setting of p-value by using SPSS version 13.0 [15].

Result

Analyzing the obtained statistical characteristics such as age, gender, and extensive wounds is in accordance with (Table 1).

Table 1: Characteristics of Subject.

Characteristics	NaCl 0.9% - honey (n=14)	NaCl 0.9% (n=14)	Value - p
Gender			0.280*
Male	13	11	
Female	1	3	
Age (years old)			0.890**
Mean	35,43 (10,61)	29,64 (11,87)	

*) Chi Square test

***) Independent Sample T Test

Based on (Table 2), note a smaller number of bacteria colonies after dilution with NaCl 0.9% and honey compared with before dilution (negative difference) is found in 12 samples and has the rank number (R1) of 78.00, while 2 samples showing the same number of bacteria colonies after and before dilution, and no samples shows a greater number of bacteria colonies after dilution (positive difference).

Table 3: Statistical test of difference between Bacterial colony before and after irrigating the wound of Open Fracture of lower leg grade IIIA with mixture of NaCl 0.9 % and Honey.

	NaCl 0.9% - madu (PostTest) - NaCl 0.9% - madu (PreTest)
Z	-3,163
Asymp. Sig. (2-tailed)	0,002

Statistical two paired sample tests were performed with SPSS, results in Z value of -3.163. The difference of two samples tested by Asymp Sig (2-tailed) obtained the value 0.002. This means that

Table 4: The difference between Bacterial colony before and after irrigating the wound of Open Fracture of lower leg grade IIIA with NaCl 0.9 % alone.

		N	Mean Rank	Sum of Ranks
NaCl 0.9% (PostTest) - NaCl 0.9% (PreTest)	Negative Ranks	13	7,31	95,00
	Positive Ranks	1	10,00	10,00
	Ties	0		
	Total	14		

Statistical two paired sample tests were performed with SPSS, results in Z value of -2.765. The difference of two samples tested by Asymp Sig (2-tailed) obtained the value 0.006. This means that there is significant difference between the data before and after the group was given NaCl 0.9% dilution (Table 5). U Mann-Whitney test was done with the help of SPSS version 13.0 app, the result is as following (Table 6).

Table 5: Statistical test of difference between Bacterial colony before and after irrigating the wound of Open Fracture of lower leg grade IIIA with NaCl 0.9 % alone.

	NaCl 0.9% (PostTest) - NaCl 0.9% (PreTest)
Z	-2,765
Asymp. Sig. (2-tailed)	0,006

Table 6: Number of Rank Group pre-test and post-test data.

	Group	N	Mean Rank	Sum of Ranks
PreTest	NaCl 0.9% - honey	14	12,57	176,00
	NaCl 0.9%	14	16,43	230,00
	Total	28		
PostTest	NaCl 0.9% - honey	14	7,50	105,00
	NaCl 0.9%	14	21,50	301,00
	Total	28		

Table 7: Statistics Test-U group pre-test and post-test data.

	PreTest	PostTest
Mann-Whitney U	71,000	0,000
Wilcoxon W	1,76,000	1,05,000
Z	-1,557	-4,885
Asymp. Sig. (2-tailed)	0,119	0,000
Exact Sig. [2*(1-tailed Sig.)]	0,227	0,000

there is significant difference between the data before and after the group was given the dilution of NaCl 0.9% and honey mixture (Table 3).

The same experiment was done using 0.9% NaCl without the mixture of honey. A smaller number of bacteria colonies after dilution with NaCl 0.9% compared than before dilution (negative difference) is found in 13 samples and has the rank number (R1) of 95.00, no samples showing the same number of bacteria colonies after and before dilution, and a greater number of bacteria colonies after dilution (positive difference) is found in 1 sample and has the rank number (R2) of 10.00 (Table 4).

There is a significant difference in the reduction in the number of colonies of bacteria in the samples that were given 0.9% NaCl and honey, as compared to the samples that were given 0.9% NaCl (Table 7). There was no significant difference between pretest data group classes that were given a mixture of NaCl 0.9% - honey and pretest data that were given NaCl 0.9% alone. But there are significant differences between post test data group classes by a mixture of 0.9% NaCl with honey-class and post-test data by NaCl 0.9%.

Discussion

The purpose of open fractures management is to restore the normal or optimal function of a limb as soon as possible. Thus, it is important to prevent the wound from infection. One of the attempts is cleansing the wound to reduce the number of bacteria colonies so it can dilute the concentration of contaminants and bacteria.

The nature of honey with a high level of osmolarity is caused by the high sugar levels which dilute the product from microbial and stop the growth of all microbial species. It contains glucose oxidase enzyme so that when the honey is diluted, it will produce a low concentration hydrogen peroxide which has an antiseptic and antimicrobial effect. Honey can also create a moist environment by reducing the lymph fluid from the wound tissue through a high osmotic work. Debridement effect of honey is caused by protease enzyme by releasing hydrogen peroxide. In this study, there are 28 patients, 14 patients underwent dilution with a mixture of 0.9% NaCl and honey, and 14 other patients underwent dilution with 0.9% NaCl alone. The data were compared and it showed that dilution with a mixture of 0.9% NaCl with honey will reduce the number of bacteria colonies significantly compared to those wounds diluted with NaCl 0.9% alone.

Conclusion

The conclusion of this study is that there is a significant difference between dilution using mixture of normal saline – honey compared to dilution with normal saline only in lowering the bacterial count.

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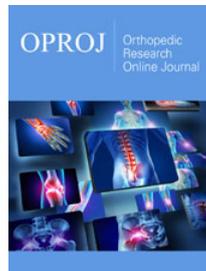
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