Effect of Vinegar Therapy on Bacterial Growth in Diabetic Foot Ulcers

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Abstract

Background: Non-healing diabetic foot ulcers is a common problem worldwide, almost 15% of all diabetic patients will develop one or more foot ulcers some of them will require amputation. Foot infections are usually caused by aerobic gram-positive bacteria in the acute cases or a mixture of aerobic gram positive, aerobic gram negative and anaerobic organisms in chronic wounds.

Objectives: To assess the efficacy of vinegar therapy on bacterial growth in the process of treating diabetic foot ulcers.

Materials and methods: Retrospective comparison of changes in bacterial culture, necrotic and total surface area of chronic wounds treated with either vinegar therapy or standard (control) surgical or nonsurgical therapy. Total of 30 patients with non-healing ulcers were divided into 3 groups; 10 wounds were treated with conventional therapy, 10 with vinegar therapy, and 10 with hypertonic saline.

Results: Repeated measures ANOVA indicated no significant change in necrotic tissue. During the first 14 days of conventional therapy, there was no significant debridement of necrotic tissue, and heavy growth of different pathogens mainly *Staphylococci*, *Streptococci*, and *Pseudomonas*. During the same period with vinegar therapy, necrotic tissue decreased by an average of 4.1 cm^2 (P = 0.02). After 3 weeks of therapy, conventionally treated wounds were still covered with necrotic tissue over 41% of their surface and still growth of different pathogens , whereas after only 3 weeks of therapy vinegar-treated wounds were completely debrided (P = 0.001) and 70% of cultures were negative . Vinegar therapy was also associated with hastened growth of granulation tissue and grater wound healing rates.

Conclusion: Vinegar therapy was more effective and efficient in debriding non healing foot and leg ulcers in diabetic patients than was continued conventional care and associated with lower bacterial growth rates.

Key words: Vinegar therapy, diabetic wound**,** *Staphylococci*,

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Introduction

Chronic non-healing wounds, such as leg ulcers, which affect up to 2% of the population continue to pose a treatment challenge to the clinician [1, 2]. Impaired wound healing is a common and costly problem for those with diabetes. Non-healing diabetic foot ulcers account for 25–50% of all diabetic hospital admissions, and most of the 60,000–70,000 yearly amputations in the U.S. [3]. Almost 15% of all diabetic patients will develop one or more foot ulcers, and 15-25% of those ultimately will require amputation [3].

Peripheral neuropathy has a central role in the development of a foot infection , the most common pathogens in acute, previously

untreated, superficial infected foot wounds in patients with diabetes are aerobic gram-positive bacteria, particularly *Staphylococcus aureus* and beta-hemolytic Streptococci (group A, B, and others)[4]. Infection in patients who have recently received antibiotics or who have deep limb-threatening infection or chronic wounds are usually caused by a mixture of aerobic gram positive, aerobic gram negative (e.g., *Escherichia coli*, *Proteus* species, *Klebsiella* species), and anaerobic organisms(e.g., *Bacteroides* species, *Clostridium* species, *Peptococcus* and *Peptostreptococcus* species) [4].

Anaerobic bacteria are usually part of mixed infections in patients with foot ischemia or gangrene [5]. Methicillin-resistant *S. aureus* (MRSA) is a more common pathogen in patients who have been previously hospitalized or who have recently received antibiotic therapy, MRSA infection can also occur in the absence of risk factors because of the increasing prevalence of MRSA in the community [6, 7].

As the development of bacterial resistance to antibiotics continues, the need for new antimicrobial agents has led to reemergence of therapies that have been used for centuries but have become less fashionable during the antibiotic era whom are safe and broadly effective and have low propensity to induce resistance. Debridement involves the removal of devitalized and contaminated tissue from the wounds to expose healthier tissue and facilitate healing [8].

Vinegar debridement therapy was used in many hospitals around the world for treating bone and soft-tissue infections due to its antimicrobial properties, meanwhile, it has been used as an antibiotic for the dressing of wounds as well as other uses, so that the vinegar has been suggested as a cure or ingredient in a cure for most human and many animal ailments [9, 10].

However, no large prospective clinical trials have been conducted for vinegar therapy to support these information's recently. The present study was designed to assess the utility of vinegar therapy on diabetic foot ulcer through its activity as antimicrobial agent. We analyze the clinical course and outcomes of a cohort of diabetic patients whose foot and leg ulcers were treated with standard versus vinegar therapy, and obtain aerobic and anaerobic cultures at different periods throughout these treatments.

Materials and methods

Patient's selection

A total of thirty diabetic patients were recruited in the study. All patients have non healing foot and leg wounds were already monitored in service at Almadaen general hospital, for at least 2 weeks, were found to have contours that could be measured by planimetry, making them eligible for this study.

Patients were divided into three groups; (1) 10 patients were received conventional antibiotic therapy, (2) 10 patients were received hypertonic saline, (3) 10 patients were received **v**inegar therapy.

Wound evaluations, Bacterial isolation and antibiotic therapy

Ulcer length, width, circumference, and surface area were calculated from digitized photographic images. Primary outcome measures included (according to Ingrid k 2006 [11].

1) change in relative and absolute amounts of necrotic tissue (defined as non-perfused, nonviable soft tissue); *2*) change in relative amounts of granulation tissue (defined as viable, well-vascularized, undifferentiated tissue); *3*) change in wound surface area over time; *4*) the length of time until complete wound healing;5) culture and sensitivity methods. Initial empiric antibiotic therapy is based on the severity of the infection, history

of recent antibiotic treatment, previous infection with resistant organisms, recent culture results, current Gram stain findings, and patient factors (e.g., drug allergy **,**twice weekly culture using wound swab in different kinds of treatment, were done using transport media swab, each specimen inoculated on two blood agar plates and two chocolate agar plates for aerobic and anaerobic bacteria, incubated at 37^{o}c for 48 hours. Colony morphology, Grams stain and biochemical examination (using api 20 test strip) were used for bacterial diagnosis. Wound was daily washed and wound debridement and dressing using vinegar versus other conventional methods were done.

Statistical analysis

Normally distributed ordinal and interval data were analyzed using Student's *t*-test or logistic regression when variance was equal, and Welch's *t* test when variance was not equal. Ordinal and interval data not normally distributed were evaluated using the Mann-Whitney *U* test. Nominal data were analyzed using Pearson's χ^2 test (except when less than five cases were expected, thereby invoking Fisher's exact test). Changes in tissue quality and surface area over time were evaluated using repeated measures ANOVA. The hypothesis of equality of means was discarded when the probability (*P*) of a type I error was ≤5%. Analyses were performed with SPSS statistical software (SPSS, Chicago, IL).

Results

Differences between three groups of patients receiving different kinds of treatment were described in table 1. As shown in table 1 and figure one no clear difference in ulcer sizes were observed during the first week of conventional, hypertonic saline, and vinegar therapy in the three study groups, while obvious changes in ulcer size observed in vinegar therapy compared with the conventional group(P=0.001) and(P=0.014)in hypertonic saline against conventional group. The correlation between vinegar therapy against hypertonic saline did not reach the statistical significance (P= 0.37).

Table (1): Different ulcer sizes subjected to different kinds of treatment methods and changes in ulcer sizes up to three weeks

	Method of treatment			
Ulcer size	**Conventional method (n=10)**	**Hypertonic saline (n=10)**	**Vinegar therapy (n=10)**	**P (ANOVA)**
at baseline (pretreatment)				0.66[NS]
Range	(2 to 8)	(3 to 8)	(3 to 7)	
Mean	5.2	5.4	4.7	
SD	2.0	1.8	1.3	
after 3 weeks				****
Range	(0.5 to 6.4)	(1 to 5.2)	(0 to 3.3)	
Mean	3.65	3.02	2.03	
SD	1.9	1.3	1.1	
changes after 3 weeks compared to baseline				0.004
Range	(-2.5 to -1)	(-3.6 to -1.6)	(-3.7 to 0)	
Mean	-1.55	-2.38	-2.67	
SD	0.4	0.6	1.0	
P (LSD) for difference in mean between				
Hypertonic saline X conventional group = 0.014				
Vinegar and conventional group = 0.001				
Vinegar X Hypertonic saline group = 0.37[NS]				

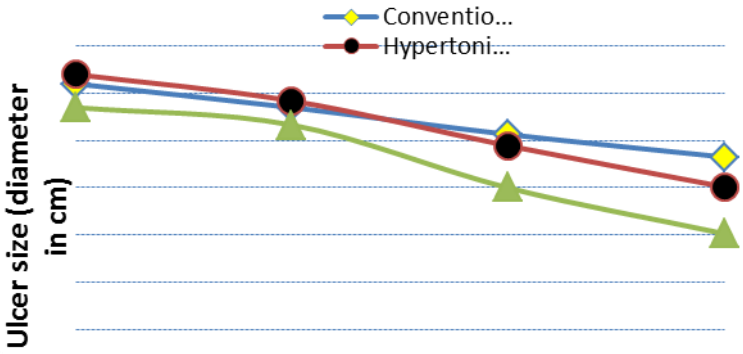

Figure (1): Diagrammatic illustration of ulcer sizes during therapy.

The 70%negative culture results after three weeks of vinegar therapy and decreased growth rates of different bacterial isolates specially the micro-aerophilic and non-aerobic ones assist the benefit of vinegar and its treatment efficacy over other treatment methods (table 2, 3). On the other hand decrease bacterial growth and negative cultures (40%) also noticed among conventional treatment after 3 weeks of therapy (table 3).

Table (2): Twice weekly culture using wound swab in different kinds of treatment.

	Method of treatment					
	Conventional method		**Hypertonic saline**		**Vinegar therapy**	
Results of culture	**N**	**%**	**N**	**%**	**N**	**%**
at baseline (pretreatment)						
Staphylococci.	6	60.0	3	30.0	5	50.0
Streptococci	0	0.0	1	10.0	0	0.0
Enterobacteriacae	2	20.0	2	20.0	1	10.0
Pseudomonas	2	20.0	1	10.0	2	20.0
Eenterococcus	0	0.0	1	10.0	1	10.0
Bacteroides	0	0.0	0	0.0	1	10.0
Peptostreptococcus	0	0.0	2	20.0	0	0.0
Total	10	100.0	10	100.0	10	100.0
after 3 weeks						
Negative	0	0.0	4	40.0	7	70.0
Staphylococci.	3	30.0	2	20.0	2	20.0
Streococcipt	2	20.0	2	20.0	1	10.0
Enterobacteriacae	1	10.0	0	0.0	0	0.0
Pseudomonas	2	20.0	0	0.0	0	0.0
Eenterococcus	1	10.0	1	10.0	0	0.0
Bacteroides	1	10.0	1	10.0	0	0.0
Total	10	100.0	10	100.0	10	100.0

Table (3): Positive culture comparison between different methods of treatment.

	Method of treatment						
	Conventional method (n=10)		Hypertonic saline (n=10)		Vinegar therapy (n=10)		P (Chi-square)
Positive culture	**N**	**%**	**N**	**%**	**N**	**%**	
At baseline (pretreatment)	10	100.0	10	100.0	10	100.0	
after 1 week	10	100.0	10	100.0	10	100.0	
after 2 weeks	10	100.0	10	100.0	10	100.0	
after 3 weeks	10	100.0	6	60.0	3	30.0	0.005
Treatment efficacy		0%		40%		70%	
P Chi-square for difference between							
Normal saline X conventional group = 0.09[NS]							
Vinegar and conventional group = 0.003							
Vinegar X Normal saline group = 0.37[NS]							

Table 4 show that vinegar therapy was associated with faster debridement and good healing rates of ulcers beginning from the second week of treatment (60%) reaching (100%) in the third one which was higher than other methods. Vinegar treated wounds show significant changes in necrotic tissue sizes after three weeks of treatment (table 5 and figure 2).

Table (4): Ulcer healing compared in different types of therapies.

Subjective assessment of healing	Method of treatment					
	Conventional method		Hypertonic saline		Vinegar therapy	
	N	**%**	**N**	**%**	**N**	**%**
after 1 week						
Negative	10	100.0	10	100.0	10	100.0
Mild	0	0.0	0	0.0	0	0.0
Good	0	0.0	0	0.0	0	0.0
Total	10	100.0	10	100.0	10	100.0
after 2 weeks						
Negative	2	20.0	0	0.0	0	0.0
Mild	8	80.0	6	60.0	4	40.0
Good	0	0.0	4	40.0	6	60.0
Total	10	100.0	10	100.0	10	100.0
after 3 weeks						
Negative	0	0.0	0	0.0	0	0.0
Mild	9	90.0	4	40.0	0	0.0
Good	1	10.0	6	60.0	10	100.0
Total	10	100.0	10	100.0	10	100.0
Mean rank	8.5		16.0		22.0	
P (Kruskall-Wallis) for difference in median healing grade between the 3 groups < 0.001						
P (Mann-Whitney) for difference in median healing grade between:						
Normal saline X conventional group = 0.022						
Vinegar and conventional group <0.001						
Vinegar X Normal saline group = 0.029						

Table (1): Necrotic tissue sizes compared in different therapy methods.

	Method of treatment			
Necrotic tissue diameter (cm)	**Conventional method (n=10)**	**Hypertonic saline (n=10)**	**Vinegar therapy (n=10)**	**P (ANOVA)**
at baseline (pretreatment)				0.65[NS]
Range	(1 to 6)	(1 to 7)	(1 to 6)	
Mean	3.9	4.2	3.5	
SD	1.7	1.8	1.5	
after 3 weeks				****
Range	(0.2 to 4.3)	(0 to 4.1)	(0 to 2.5)	
Mean	2.26	1.93	0.92	
SD	1.4	1.3	0.9	
changes after 3 weeks compared to baseline				0.1[NS]
Range	(-2.5 to -0.8)	(-4 to -1)	(-4 to -0.5)	
Mean	-1.64	-2.27	-2.58	
SD	0.5	0.8	1.4	

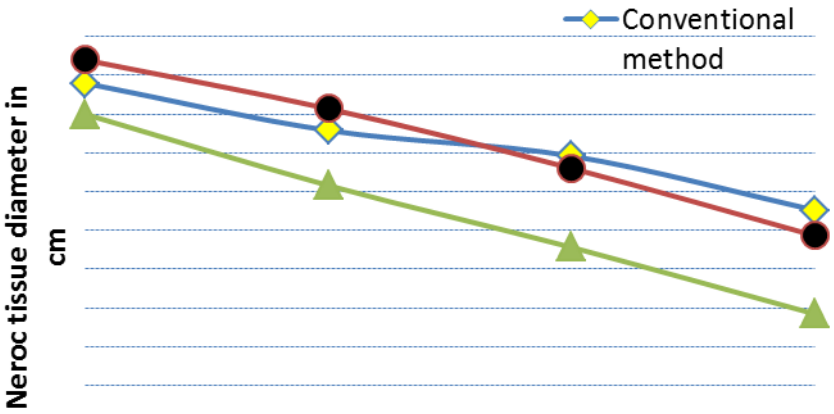

Figure (2): Diagramatic illustration of necrotic tissue size in different therapy.

However the changes in the wound PH after 3 weeks of treatment was observed with vinegar (PH=4-7) when compared with other treatment methods (PH= 6-9) table 6.

Table (2): Wound PH changes in different therapies.

	Method of treatment			
	Conventional method (n=10)	**Hypertonic saline (n=10)**	**Vinegar therapy (n=10)**	**P (ANOVA)**
PH-after 3 weeks				<0.001
Range	(6 to 9)	(6 to 9)	(4 to 7)	
Mean	7.4	7.1	5.1	
SD	0.8	0.9	1.0	
P (LSD) for difference in mean between				
Hypertonic saline X conventional group = 0.47[NS]				
Vinegar and conventional group <0.001				
Vinegar X Hypertonic saline group <0.001				

Discussion

There has not been a study comparing vinegar therapy to conventional treatments for diabetic foot wounds. The present analysis demonstrated that vinegar therapy is more effective and efficient in debriding non healing foot and leg ulcers in diabetic patients than the typical conventional treatment currently prescribed.

Vinegar therapy was also associated with a more rapid decrease in wound size, decrease bacterial growth and increase in granulation tissue, making the wounds ready for surgical closure. The higher number of patients actually achieving complete wound closure within the 3-week study period (14% with vinegar therapy vs. 0% with conventional therapy) did not reach statistical significance.

It has been found that lowering the pH of ulcer to more acidic environment, by using vinegar, decreases the bacterial growth and activity especially for the microaerophilic organisms and sequentially accelerates healing of ulcers and reduces necrotic tissue size. This finding agree with Kaufman *et al* (1985) and Georgina study in *2007* who reported that any factor that could cause a small change in the pH of the wound may appreciably alter the available supply of oxygen to the ulcerated tissues thus preventing the permanent obstacles to the transport of oxygen in chronic recurrent wound which can harbor at least four different types of bacteria at any one time. Also agree with study of Greener *et al* (2005) who concluded that the presence of necrotic and devitalized tissue in the wound causes an increased metabolic load resulting in tissue hypoxia and excessive breakdown of extracellular matrix (ECM) by means of proteases which are not only produced by the wound itself but also as bacterial end products, this occur more rapidly in alkaline environment.

Another study by Molan (2002) and Gethin *et. al.* (2006) found that lowering the pH to more acidic have many effects like reducing the toxicity of bacterial end products, as ammonia, enhancing the abnormal collagen destruction in the ulcer bed , promotion of angiogenesis , increased macrophage and fibroblast activity and control the enzymatic activity. For this reason vinegar therapy may reduce the use of antibiotics required for providing coverage against some aerobic and anaerobic bacteria specially for hospitalized patients with limb threatening infections, this finding agree with Georgina study 2007 [13]. Who stated that using acetic acid has variable efficacy on managing the bacterial burden of the wound although it has some limitation due to its short duration of effect.

Our findings support the benefits of vinegar therapy claimed by earlier authors as Dissemond *et al* (2003) [17]. Who reported effective debridement for non-healing wounds in 22 diabetic patients treated with an average of six vinegar treatments over the course of 2 weeks; 12 wounds were debrided within just 1 week. Leung *et al* [9] and Bowler *et al* [11] similarly found vinegar therapy to be a valuable treatment for debriding diabetic foot wounds. However, reported outcomes were subjective, there were no control groups, and the effects on wound closure were not evaluated. Only two prior studies of vinegar therapy incorporated control groups. Wayman *et al* [18] demonstrated that vinegar therapy was associated with more rapid debridement and reduced cost when compared to hydro gel for the treatment of venous stasis ulcers. Neither of these prospective studies evaluated diabetic foot ulcers.

Many questions remain unanswered, and a large prospective evaluation is warranted. Although VT debrided wounds, decreased their size, and prepared them for closure more rapidly than did conventional therapy,

the rate of wound closure was not significantly higher than that associated with standard therapy. A larger study, preferably with subjects whose disease is not as advanced, might better demonstrate the impact of vinegar therapy on complete wound closure. In addition to issues of efficacy and safety, future studies also must address the cost-effectiveness of VT.

In conclusion, Vinegar therapy was more effective and efficient in debriding non healing foot and leg ulcers in diabetic patients than was continued conventional care and associated with lower bacterial growth rates.

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