



## EFFECT OF NEGATIVE PRESSURE WOUND THERAPY ON THE BACTERIAL FLORA OF WOUNDS: A PROSPECTIVE RANDOMIZED STUDY

### General Surgery

**Dr. Rishi Dhawan** Consultant, Department of Plastic and Reconstructive Surgery, Max Superspeciality Hospital, Mohali, Punjab, India

**Dr. Asif Mian Ansari\*** DNB Resident, Department of General Surgery, Max Superspeciality Hospital, Mohali, Punjab, India \*Corresponding Author

### ABSTRACT

20 patients were treated with negative pressure wound therapy (NPWT) and 20 patients by cotton gauze dressings. Bacterial cultures were done on 0, 4th and 8th day. The bacterial inoculates were compared between these two groups. NPWT was found to decrease pseudomonas species more than the S. aureus or E. coli species. The number of isolates of Pseudomonas decreased from day 0 (n=7) to day 8 (n=2) by NPWT. In the gauze dressing group, the number of isolates marginally increased from day 0 (n=5) to day 4 (n=6) and then decreased to the baseline at day 8 (n=5). The number of isolates of E coli under gauze dressings also decreased from day 0 (n=12) to day 8 (n=6). Under NPWT however, lead an only marginal decrease in the number of isolates of E coli from day 0 (n=7) to Day 8 (n=6). Thus, NPWT is more effective to control obligate aerobic bacteria.

### KEYWORDS

NPWT, gauze dressing, Bacterial culture.

### INTRODUCTION:

Dressings have undergone a sea change in the modern world. Development from traditional wound dressings, regarded as passive dressings, that protect the wound while it heals like gauze, saline soaked gauze and tulle dressings to newer interactive dressings which modulate the environment to keep the wound environment moist and promote healing. These include collagen, films, foams, alginates hydrocolloids, and hydrogels. A more aggressive approach had lead to the development of active dressings. These include skin grafts, cellular suspensions, and growth factors.<sup>1</sup> Negative pressure wound therapy (NPWT) is a form of active dressing and acts through various mechanical and biochemical mechanisms like increased blood flow, mechanical strain, reduction in bioburden and removal of edema, toxins and matrix metalloproteinases.<sup>2-4</sup> This change in the wound environment may lead to inhibition or facilitation of growth of the different bacteria. This form of wound therapy was noticed after convincing studies on animal and later on human models by Morkwas et al which have been widely cited and acknowledged.<sup>2</sup>

The present study was undertaken to see the change of bacterial flora under NPWT and compared it with saline gauze dressings.

### MATERIAL AND METHODS

A total of 40 patients were enrolled for the study from May 2012 to November 2013. A pre-informed written consent for the study was taken from all patients. The patients had wounds that could not be closed primarily and were eligible for treatment with both open wound dressings and by NPWT. Twenty patients were non-diabetic and twenty were diabetic. These were further divided into two groups of 10 patients each- of those undergoing NPWT and those undergoing normal saline gauze dressings. Only wounds which were devoid of any necrotic material were subjected for the study.

All patients undergoing the study continued to receive antibiotics depending upon the bacterial isolate and its antibiotic sensitivity pattern.

**The technique of NPWT dressing:** The wounds were cleaned with normal saline and then filled with a polyurethane (PU) foam, with a suction catheter tunneled into the foam. The dressing was then be covered with a transparent sticking plastic drape to make it airtight. The suction catheter was connected with sterile tubing to an NPWT machine. Continuous negative pressure<sup>9</sup> of 125 mmHg was applied to the wounds.

**The technique of gauze dressing:** The wound were cleaned with normal saline and covered with moist normal saline cotton gauze and then by a cotton pad and bandaged.

**Technique of sampling:** Qualitative tissue cultures were taken from the wounds using a 5mm biopsy forceps. One Pre-therapy culture sample (Q1) was taken from all wounds before instituting negative pressure dressings or the gauze dressings considering this as day 0 of the study. The wounds with conventional dressings were dressed every day and those undergoing NPWT had a change of dressing every 4th day. After institution of NPWT or gauze dressings, a second sample was taken on the 4th day (Q2), when the dressings are routinely changed. A third sample was taken on day 8 (Q3). The tissue samples for cultures were taken as per the same schedule for both diabetic and non-diabetic patients.

**The technique of processing the Cultures:** One blood agar and the Mac-Conkey agar culture plates were incubated under aerobic conditions for 24-48 hrs. The organisms growing on the Blood agar and Mac-Conkey agar plates were identified on basis of Gram's stain, colony morphology, and standard biochemical reactions.

**Statistical analysis:** The results of the study were analyzed using unpaired 't' test for age and gender distribution and evaluation of quantitative cultures. Z test was used for comparing the topography of wounds and the number of inoculates in the various cultures. p values were calculated were considered significant if p < 0.05.

### OBSERVATIONS AND RESULTS:

**Demography:** Of the 40 patients enrolled for the study males were the predominant sex. This gender predominance was there in both the treatment groups of NPWT and the Gauze Dressings. Males formed 95% of total patients in NPWT and 85% of the total patients in the gauze dressing group. The mean age of patients undergoing NPWT was 51.60 years and in gauze dressings was 55.50 years in the diabetic. In non-diabetic patients, the mean ages were 33.2 years and 36 yrs respectively in patients undergoing NPWT and gauze dressings respectively.

The number of bacterial isolates in the patients in day 0, day 4 and day 8 cultures are demonstrated in table 1.

The number of isolates of Pseudomonas decreased from day 0 (n=7) to day 8 (n=2) under the NPWT. In the gauze dressing group, the number of isolates marginally increased from day 0 (n=5) to day 4 (n=6) and then decreased to the initial baseline at day 8 (n=5)

NPWT also lead to a decrease in the number of Acinetobacter isolates cultured from tissue samples at day 4 (n=6) and then at day 8 (n=4). No significant change in the number of isolates of Acinetobacter was seen in the gauze dressings group. The number of isolates after gauze dressings decreased in the sample at day 4 (n=6) followed by an increase again in day 8 samples to the baseline of day 0 (n=7).

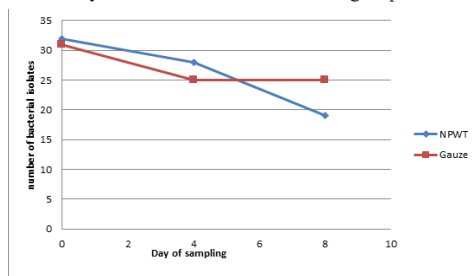
**Table 1: Bacterial profile in NPWT and gauze dressings**

Organisms	Day 0 cultures (Q1)			Day 4 cultures (Q2)			Day 8 cultures (Q3)		
	NPWT	Gauze	p-value	NPWT	Gauze	p-value	NPWT	Gauze	p-value
	No.	No.		No.	No.		No.	No.	
Organisms									
Acinetobacter	7(21.9)	7(22.6)	0.849	6(24.1)	6(24)	0.958	4(26.3)	7(26.9)	0.827
Citrobacter	0(0)	0(0)	NA	0(0)	0(0)	NA	0(0)	1(3.8)	0.187
<i>E. coli</i>	7(21.9)	12(38.7)	0.113	7(24.1)	6(24)	0.958	6(36.8)	6(22.8)	0.133
Enterobacter	1(3.1)	0(0)	0.166	0(0)	0(0)	NA	0(0)	1(3.8)	0.187
Klebsiella	2(6.2)	3(9.7)	0.328	2(6.9)	5(20)	0.115	2(10.5)	4(15.2)	0.315
Non hemolytic streptococcus(NHS)	0(0)	1(3.2)	0.161	0(0)	0(0)	NA	0(0)	0(0)	NA
Proteus	4(12.5)	2(6.5)	0.202	3(10.3)	0(0)	0.100	1(5.3)	0(0)	0.142
Pseudomonas	7(21.9)	5(16.1)	0.284	6(20.7)	6(24)	0.567	2(10.5)	5(19)	0.194
Staphylococcus	4(12.5)	1(3.2)	0.121	4(13.8)	2(8)	0.244	4(10.5)	2(7.6)	0.571
Total	32(100)	31(100)	NA	28(100)	25	NA	19(100)	26(100)	NA

On studying *S. aureus* colonies there was no change found in the number of isolates of *S. aureus* under the NPWT however the samples from wounds under gauze dressings showed a marginal increase (n=1 to n=2) between day 0 and day 4 samples and thereafter remained constant.

The number of isolates of *E. coli* under gauze dressings also decreased from day 0 (n=12) to day 8 (n=6). Under NPWT however, there was an only marginal decrease in the number of isolates of *E. coli* from day 0 (n=7) to Day 8 (n=6).

Poly-microbial versus mono-microbial growth (Figure 1): Most of the wounds studied had polymicrobial infections. The number of different bacterial species decreased progressively in both the forms of treatment but they decreased more in the NPWT group.

**Figure 1: Number of isolates: NPWT versus gauze**

Twenty patients under treatment by NPWT had 32 different bacterial isolates in the day 0 samples which reduced to 19 in day 8 tissue samples. The different bacterial isolates in the 20 patients under gauze dressings group decreased from 31 at day 0 to 25 in day 8 samples. The number of isolates decreased significantly in patients under NPWT from Day 0 samples today 8 samples ( $p=0.04$ ).

## DISCUSSION:

In our study we have tried to ascertain one of the proposed mechanisms associated with increased healing rates of NPWT, that is a decrease in the bio-burden of the wounds. Only a few other studies are prospective randomized studies on this subject.<sup>10,11,12</sup>

As the NPWT changes the wound environment there are changes expected in the bacterial flora as this changed environment may promote or inhibit the growth of different bacteria. This was demonstrated by Moues et al in 2004 who observed a comparatively larger decrease in *Pseudomonas aeruginosa* isolates (strict aerobe), than in *S. aureus* which is a facultative anaerobe.<sup>10</sup> Chester et al in a case report mentioned anaerobic septicemia following NPWT and reasoned that the microaerophilic environment may promote the proliferation of anaerobic bacteria.<sup>13</sup>

In the present study, we studied the number of isolates of various bacteria in the wounds. We found that *E. coli* was the most common bacterium isolated in diabetic patients (n=10, 31%) and *Acinetobacter* was the most common bacterium isolated in non-diabetic patients studied (n=9, 27%).

In our study, we also observed a prominent decrease in the number of *Pseudomonas* and *Acinetobacter* species in wounds under NPWT, progressively as compared to gauze dressings. These results were

similar in both the diabetic and the non-diabetic subgroups when studied separately. The reason could be that both these bacteria are obligate aerobes and their growth is hampered by oxygen-poor environment produced by NPWT. The decrease was although not found to be statistically significant.

A similar observation was also made by Boon et al in their experimental study of the effect of NPWT on wounds in porcine models.<sup>14</sup> Quantitative cultures showed a peak at day 4 followed by a drop on day 7. Khasram et al showed an increase in bacterial bio-burden during NPWT in venous leg ulcers. But this study again had a small sample size (n=6) and used a wound swab method of culture. Also, it studied only non infected venous leg ulcers.<sup>15</sup>

On the contrary, the number of isolates of facultative anaerobes such as *S. aureus* and the *E. coli* decreased more in gauze dressings than in NPWT. The number of isolates of *E. coli* decreased and were found to become significantly less following gauze dressings than that in NPWT in day 8 samples in diabetic patients with gauze dressing ( $p=0.008$ ). Thus it was seen that the oxygen-poor environment lead to a decrease in the numbers of obligate aerobes like *pseudomonas* to a greater degree than the facultative anaerobes. It was also noted that there was a significant decrease in the number of different species of bacteria in wounds under NPWT. More of polymicrobial infected wounds were converted to monomicrobial wounds under NPWT as compared to gauze dressings.

Our study had limitations of size, as the total numbers of isolates of various bacteria were small. Also, the patients under the study were administered antibiotics based on culture sensitivity of the bacterial isolate during the course of the study. Although this was done in all studied groups, it might have influenced the result.

## CONCLUSION:

We conclude that the negative pressure wound therapy leads to an accelerated decrease in the number of isolates of *Pseudomonas* (obligate aerobic bacteria) when compared to the *S. aureus* (facultative anaerobes). Also, the number of different species of bacteria infecting a wound decreased significantly more under NPWT as compared to those under gauze dressings.

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