Effect of vacuum sealing drainage combined with intermittent insulin flushing on expression of inflammatory factors and apoptosis factors in diabetic lower limb ulcer wound

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ARTICLE INFO

Article history:
Received 29 Jan 2018
Received in revised form 8 Feb 2018
Accepted 16 Feb 2018
Available online 28 Feb 2018

Keywords:
Diabetic lower limb ulcer
Vacuum sealing drainage
Intermittent insulin flushing
Inflammatory factor
Apoptosis factor

ABSTRACT

Objective: To discuss the effect of vacuum sealing drainage combined with intermittent insulin flushing on the expression of inflammatory factors and apoptosis factors in diabetic lower limb ulcer wound. Methods: A total of 48 patients with diabetic lower limb ulcer who were treated in the Endocrine Department of the hospital between January 2016 and January 2017 were divided into the control group and research group by random number table, 24 cases in each group. Control group received vacuum sealing drainage, and research group received vacuum sealing drainage combined with intermittent insulin flushing. The differences in the expression of inflammatory factors and apoptosis genes in the wound exudate were compared between the two groups before and after treatment. Results: Before treatment, the differences in the inflammatory factor contents and apoptosis gene expression in wound exudate were not statistically significant between the two groups of patients. After treatment, inflammatory factors IL-6, IL-1β, TNF-α, CRP, VCAM-1 and FGF2 contents in wound exudate of research group were lower than those of control group; apoptosis gene Bcl-2 mRNA expression in wound exudate of research group was higher than that of control group while Bax, p53 and Fas mRNA expression were lower than those of control group. Conclusions: Vacuum sealing drainage combined with intermittent insulin flushing can effectively reduce the local inflammatory response and promote the granulation cell proliferation in wound of patients with diabetic lower limb ulcer.

I. Introduction

Diabetic foot is the most common clinical complication in diabetic patients with poor blood glucose control, the patient's symptoms vary from neurological changes to the serious ulcers, infections, etc., and it can result in amputation and other serious consequences if not treated in time[1,2]. Active control of blood glucose and regular wound dressing are the routine treatments for patients with diabetic lower limb ulcer, but a large number of patients have difficult wound healing and even repeated infection. Vacuum sealing drainage (VSD) is a new treatment that uses polyvinyl alcohol hydration alginate foam dressings containing drainage tube to cover the local skin wound, adopts biological semipermeable membrane to seal it and form a relatively closed space, and uses controllable negative pressure to promote wound healing, and it has been successfully applied in the treatment of multiple traumatic infectious skin lesions[3,4]. At the same time, some scholars have pointed out that using proper solution to completely remove the necrotic exfoliated material from the wound when VSD is adopted could create more favorable conditions for the subsequent cell growth and tissue healing. In this study, VSD was used together with intermittent insulin flush for the treatment of patients with diabetic lower extremity ulcer, and the role of the method in reducing the local inflammation and promoting tissue healing was discussed to lay the practical basis for selecting therapies for similar patients in the future.
2. Materials and methods

2.1. Case information

A total of 48 patients with diabetic lower limb ulcer who were treated in the Endocrine Department of the hospital between January 2016 and January 2017 were divided into the control group (n=24) and research group (n=24) by random number table. Control group included 14 males and 10 females, they were 47-71 years old, and the course of diabetes was 6-19 years; research group included 15 males and 9 females, they were 49-70 years old, and the course of diabetes was 8-18 years. The distribution of above baseline data was not significantly different between the two groups, the follow-up data were comparable, and the study was approved by the hospital ethics committee.

Inclusion criteria: (1) diagnosed with type 2 diabetes; (2) combined with lower limb ulcers; (3) without injury caused by lower limb trauma; (4) cooperating with completing the whole treatment without dropping out. Exclusion criteria: (1) combined with systemic infectious diseases caused by other reasons; (2) combined with serious autoimmune diseases; (3) combined with malignant neoplastic diseases.

2.2. Therapy

Both groups of patients were treated with conventional hypoglycemic therapy by insulin, and control group received vacuum sealing drainage, which was as follows: disposable VSD material was used to seal the wound, the suction tube was connected to the vacuum device, continuous vacuum suction (negative pressure -60~50 kPa) was done, there were visible VSD foam collapse, no subdural effusion and fluent suction tube, and the suction lasted for 6 d. Research group received vacuum sealing drainage combined with intermittent insulin flush, which was as follows: insulin (1 U/10 mL) was added in saline syringe, it was connected to the VSD flushing tube, the solution was pumped at 40 mL/h, once/d for 8 h each time, and the treatment lasted for 6 d.

2.3. Inflammatory factors

Before and after treatment, the wound exudate specimens were obtained from all patients, and enzyme-linked immunosorbent assay method was used to determine the contents of inflammatory mediators interleukin-6 (IL-6) and interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1) and fibroblast growth factor 2 (FGF2).

2.4. Apoptosis factors

Before and after treatment, the wound exudate specimens were obtained from the two groups, fluorescence quantitative PCR method was adopted to determine the apoptosis gene expression, and the specific steps were as follows: cracking cells → take the upper water phase after centrifugation → precipitating total RNA gel block, and cleaning and drying it → synthesizing sample cDNA (reverse transcription kit) → amplifying apoptosis genes Bel-2, Bax, p53 and Fas with fluorescence quantitative PCR kit. The target gene band was obtained in computer software, and the mRNA expression was semi-quantitatively calculated.

2.5. Statistical processing

Inflammatory mediators and apoptosis factors were all expressed as mean ± standard deviation and compared by t test. The software in the study was SPSS 25.0 and the differences in obtained statistics were statistically significant if P<0.05.

3. Results

3.1. Inflammatory mediators

Before treatment, IL-6, IL-1β, TNF-α, CRP, VCAM-1 and FGF2 contents in wound exudate were not significantly different between the two groups of patients (P>0.05). After treatment, IL-6, IL-1β, TNF-α, CRP, VCAM-1 and FGF2 contents in wound exudate of both groups were lower than those before treatment; IL-6, IL-1β, TNF-α, CRP, VCAM-1 and FGF2 contents in wound exudate of research group were lower than those of control group (P<0.05), shown in Table 1 and 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>IL-6 (pg/mL)</th>
<th>IL-1β (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>CRP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>24</td>
<td>15.38±1.79</td>
<td>9.35±0.98</td>
<td>23.73±2.85</td>
<td>8.29±0.92</td>
</tr>
<tr>
<td>Research group</td>
<td>24</td>
<td>15.67±1.68</td>
<td>5.75±0.62</td>
<td>18.94±2.15</td>
<td>8.31±0.99</td>
</tr>
<tr>
<td>p</td>
<td>0.218</td>
<td>0.091</td>
<td>0.055</td>
<td>0.055</td>
<td>0.055</td>
</tr>
</tbody>
</table>

Table 1.
Comparison of inflammatory mediator contents in wound exudate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>VCAM-1 (ng/mL)</th>
<th>FGF2 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>24</td>
<td>1.204±0.457</td>
<td>19.82±2.53</td>
</tr>
<tr>
<td>Research group</td>
<td>24</td>
<td>1.215±0.338</td>
<td>19.46±2.64</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table 2.
Comparison of inflammatory mediator contents in wound exudate.

Note: compared with same group before treatment, P<0.05.
Table 3.
Comparison of apoptosis factor contents in wound exudate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bcl-2 Before treatment</th>
<th>Bcl-2 After treatment</th>
<th>Bax Before treatment</th>
<th>Bax After treatment</th>
<th>p53 Before treatment</th>
<th>p53 After treatment</th>
<th>Fox Before treatment</th>
<th>Fox After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>24</td>
<td>99.27±10.21</td>
<td>118.47±13.42</td>
<td>101.74±13.28</td>
<td>82.44±9.17</td>
<td>98.64±10.32</td>
<td>79.25±6.83</td>
<td>100.77±13.42</td>
</tr>
<tr>
<td>Research group</td>
<td>24</td>
<td>101.88±13.42</td>
<td>136.91±5.88</td>
<td>99.85±11.63</td>
<td>61.88±7.49</td>
<td>100.95±13.24</td>
<td>54.95±7.18</td>
<td>101.85±12.47</td>
</tr>
<tr>
<td>p</td>
<td>0.271</td>
<td>9.263</td>
<td>0.194</td>
<td>11.751</td>
<td>0.452</td>
<td>8.989</td>
<td>0.198</td>
<td>9.273</td>
</tr>
<tr>
<td>p*</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Note: compared with same group before treatment, "p<0.05.

3.2. Apoptosis factors

Before treatment, Bcl-2, Bax, p53 and Fox mRNA expression in wound exudate were not significantly different between the two groups of patients (p>0.05). After treatment, Bcl-2 mRNA expression in wound exudate of both groups were higher than those before treatment while Bax, p53 and Fox mRNA expression were lower than those before treatment; Bcl-2 mRNA expression in wound exudate of research group was higher than that of control group while Bax, p53 and Fox mRNA expression were lower than those of control group (p<0.05), shown in Table 3.

4. Discussion

Long-term poor blood glucose control in diabetic patients may lead to peripheral neuropathy and peripheral vascular diseases, and when they are united and combined with excessive mechanical stress (from shoes, ground, adjacent toes, etc.), foot soft tissue damage can appear and cause tissue ulcer infection. The patients with diabetic foot lose the skin functions of normal sweating and temperature adjustment, which blocks the rehydration of local tissues, and results in further tissue destruction and the bacterial colonization in the deep tissue. Continuous progress of diabetic foot can lead to the complete loss and even necrosis of the lower limb function in the lesion, and is the primary cause of clinical non-traumatic amputation. Active control of blood glucose is the basis of diabetic foot treatment, but local wound healing depends on other surgical methods. The VSD device can transform open wound into a closed state, and has the following advantages: (1) blocking external bacteria invasion; (2) eliminating dead space and removing interstitial effusion via negative pressure; (3) forming the physical traction to the wound base via negative pressure, and stimulating the proliferation of fibroblasts. At present, some scholars put forward that at the same time of VSD treatment, intermittent flushing with flushing fluid is added to further clean the wound and promote tissue healing, insulin can activate immune cells and strengthen the cytokine chemotaxis through phosphatidylinositol 3-kinase /protein kinase B pathways, it plays a positive role in improving the local wound inflammation, promoting granulation and other aspects, and therefore, insulin flushing fluid combined with VSD device was used in the treatment of patients with diabetic lower limb ulcer in this study.

Wound infection and local inflammatory response are the direct causes of lower extremity ulceration in diabetic patients, the persistent inflammatory exudate in the wound can hinder the granulation and even lead to further damage to the skin soft tissue. Previous studies have shown that IL-6, IL-1β, TNF-α, CRP and other typical pro-inflammatory factors are massively secreted in local wound tissue of diabetic foot, which can stimulate the microvascular endothelial factor release, lead to decreased blood flow in nervous tissue, eventually aggravate local autonomic nervous function damage and wound inflammation, and inhibit the healing of ulcer wound. VCAM-1 and FGF2 are new inflammatory factor, VCAM-1 belongs to the immunoglobulin superfamily and is involved in inflammation and immunity adjustment, and the endothelial cells secrete more VCAM-1 under inflammatory condition and induce local WBC and neutrophil infiltration; FGF2 is secreted by fibroblasts, and the increase of its expression is a reactive protective mechanism for the wound healing in diabetic foot patients, which can reduce the activation of ERK1/2 signaling pathway and inhibit apoptosis. The study showed that inflammatory factors IL-6, IL-1β, TNF-α, CRP, VCAM-1 and FGF2 contents in wound exudate of both groups decreased after treatment, and the decrease of above inflammatory factor contents in exudate of research group was higher than that of control group after treatment, it illustrates that the VSD combined with intermittent insulin flushing can more effectively relieve the diabetic foot ulcer wound inflammation, and this is an important mechanism for it to promote tissue healing.

The inflammatory response of diabetic foot wound is an important factor affecting its healing, while the expression of local pro-apoptosis / anti-apoptosis genes is the most direct factor determining the granulation and tissue healing progress. Bcl-2 is a suppressor gene of apoptosis, which can effectively inhibit the apoptosis induced by multiple factors. Bax belongs to the Bcl-2 family but is opposite to it in function, and its combination with Bcl-2 can promote apoptosis, so the expression of Bcl-2/Bax can greatly determine the apoptosis activity. Many studies have confirmed...
that p53 gene overexpression can promote myocardial cell and skeletal muscle cell apoptosis, and studies have also shown that p53 gene expression is consistent with the course of diabetes. Fas is also a pro-apoptotic gene, and its expression is high in the patients with diabetic peripheral neuropathy and diabetic cardiomyopathy (21). The study showed that Bel-2 expression in wound exudate of both groups increased while Bax, p53 and Fas expression decreased after treatment, and the change in above gene expression in research group was more significant after treatment, illustrating that the VSD combined with intermittent insulin flushing can more effectively inhibit granulation apoptosis and promote granulation tissue hyperplasia.

To sum up, it can be concluded that on the basis of routine hypoglycemic treatment, the VSD device combined with intermittent insulin flushing therapy can accelerate the elimination of local lower extremity wound inflammation, promote granulation cell proliferation and inhibit its apoptosis in patients with diabetic lower extremity ulcers, it helps promote wound healing, and it is worthy of popularization and application in clinical practice in the future.

References


