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Wound healing process and related laboratory indexes in patients with type 2 diabetes mellitus after hyperbaric oxygen intervention.

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Abstract

Objective: To investigate the effect of hyperbaric oxygen intervention on chronic wound healing in type 2 diabetes mellitus, and its influence on serum inflammatory factors, oxidative stress factors and angiogenesis related factors.

Methods: Totally 78 type 2 diabetes mellitus patients with chronic wounds treated in our hospital were randomly divided into 2 groups, 39 cases in each group. The control group was treated with routine surgical wound intervention, while the observation group receiving hyperbaric oxygen therapy combined with routine surgical wound intervention. The trend of the change of wound area were compared between the 2 groups. C- reactive protein (CRP), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), superoxide dismutase-1 (SOD-1), malondialdehyde (MDA), vascular endothelial growth factor (VEGF) and angiopoietin II (Ang-2) in peripheral blood before and after treatment in 2 groups were observed.

Results: In the observation group, the wound area at 2~5 weeks during treatment were significantly smaller than those in the control group (P<0.05). Before treatment, CRP, IL-6, SOD-1, TNF-α, MDA, VEGF and Ang-2 in 2 groups were close (P>0.05), in 30 days after treatment, CRP, IL-6, TNF-α and MDA in the treatment group were much less than those in the control group, SOD-1, VEGF and Ang-2 in the treatment group were much more than those in the control group, the differences were statistically significant (P<0.05).

Conclusion: Hyperbaric oxygen intervention can promote the healing of chronic wound in type 2 diabetes mellitus, which may be related to the inhibition of CRP, TNF-α, IL-6, MDA, and the promotion of SOD-1, VEGF and Ang-2.

Keywords: Hyperbaric oxygen, Type 2 diabetes mellitus, Chronic wounds, Inflammatory factors, Oxidative stress, Angiogenesis.

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Introduction

The skins in the diabetic patients are generally vulnerable to injury, and most injured skins are demonstrated as delayed wound healing or non-healing wound [1]. At present, the causes for such difficult-to-heal wounds have been not yet fully explored clearly, and they are considered to be the combined action result of multiple factors in clinics, which may involve inflammatory reaction, oxidative stress reaction and changes in biological behaviors of extracellular matrix and growth factors [2-4]. Chronic ulcers of the lower extremity pose a major health care problem, especially among individuals with diabetes. Patients with diabetes have a 3-11% annual risk of developing lower extremity ulcers [5,6]. Ischemic diabetic ulcers are notoriously difficult to treat and require complex and costly multimodal treatment, consisting of pressure offloading, optimizing glycemic control, revascularization, and local wound treatment [7]. Hyperbaric oxygen therapy is used variably in clinical practice, based on the premise that improving the oxygenation of wounds may expedite their healing [8]. Hyperbaric oxygen can promote the healing of chronic refractory wounds through increasing the oxygen concentration in plasma and tissue cells, which has been reported both at home and abroad [9-11], but there are still few reports on the treatment effect of the hyperbaric oxygen on chronic wounds in the patients with type 2 diabetes as well as its impacts on the inflammatory factors, oxidative stress factors and angiogenesis related factors. A total of 78 diabetic patients with chronic wounds were included in this study, and a controlled study was carried out to solve the above-mentioned problems. The results were reported as follows.

Materials and Methods

General information

A total of 78 type 2 diabetes patients with chronic wounds treated in the outpatient clinic of our hospital from January

2014 to June 2017 were enrolled as the subjects to carry out a randomized prospective study, and this study had been approved by the medical ethics committee in our hospital. The patients were divided into two groups using a completely randomized method. Observation group: there were 39 patients in the observation group, including 18 males and 21 females; the patients aged 34 -71 years , with an average of (53.72 ± 14.18) years; the course of diabetes was 5-11 years, with an average of (9.82 ± 4.24) years; the wound duration was 35- 52 days, with an average of (42.79 ± 4.13) days; the wound area was 6.3-11.4 cm^2, with an average of (8.94 ± 3.61) cm^2; There were 6 patients with unhealed postoperative wounds, 12 patients with unhealed limb trauma infections, 16 patients with grade II-III foot ulcer (Wanger grading), 4 patients with unhealed bullosis diabeticorum and 1 patient with superficial II degree burns. Control group: there were 39 patients in the contrlo group, including 16 males and 23 females; the patients aged 32 -74 years , with an average of (54.41 ± 18.31) years; the course of diabetes was 4-13 years, with an average of (9.18 ± 3.85) years; the wound duration was 32- 51 days, with an average of (43.18 ± 5.04) days; the wound area was 5.8-11.7 cm^2, with an average of (8.40 ± 3.13) cm^2; There were 8 patients with unhealed postoperative wounds, 11 patients with unhealed limb trauma infections, 16 patients with grade II-III foot ulcer (Wanger grading), 3 patients with unhealed bullosis diabeticorum and 1 patient with superficial II degree burns. There were no significant differences in general information between the two groups (P>0.05). This research was approved by the Ethical Committee of Beijing PLA Navy general hospital according to the declaration of Helsinki promulgated in 1964 as amended in 1996, the approval number is 2014002.

Inclusion criteria

The patients were definitely diagnosed with type 2 diabetes by referring to the standard reference "China Guideline for Prevention and Treatment of Type 2 Diabetes" [12]; the wound duration ≥ 4 weeks; the age of patients ≥ 18 years; the patients had a single wound; the patients were well informed of this study and signed a consent form.

Exclusion criteria

The patients who had cancer wounds, acute wounds, foot ulcers with severe ischemia, ankle/brachial ratio <0.7 or severe complications such as multiple organ failure were excluded.

Rejection criteria

The patients who asked to withdraw from the study, were automatically discharged or abandoned the treatment were rejected.

Wound treatment

The patients in the control group received conventional basic intervention treatment for surgical wound: in order to improve the microcirculation and systemic nutrition, fasting blood glucose was controlled to less than 8.0 mmol/L and 2 h postprandial blood glucose was controlled to less than 11.2 mmol/L through diet and drug intervention, sensitive antibiotics were administrated according to the results of bacterial culture, and timely debridement was performed, disposable negative pressure drainage material was used to cover the wound and was connected to the negative pressure device for continuous negative pressure drainage, the pressure was set as -30 to -25kPa, and then the infusion tube of saline was connected directly with the negative pressure washing tube, the patients were washed regularly at a speed of 40 drops/ min, twice a day, 150 min each time. The patients in the observation group received combined hyperbaric oxygen intervention (Hua Xin Co Ltd., Weifang, China) on the basis of the above mentioned treatment: 100% pure oxygen, pressure 243 kPa, high pressure for 15 min, low pressure for 10 min, and inhaling of oxygen for 60~70 min, taking a rest for 5 min in the course of treatment.

Observation indicators

(1) Wound healing effect: the transparent graph paper was used to measure the patient's wound area before treatment and at the end of every week during treatment. (2) Cytokines: the elbow vein blood were collected from the patients with an empty stomach before treatment and after 30 days of treatment respectively, the blood serum was extracted to detect the levels of inflammatory factors, oxidative stress indictors and angiogenesis related factors. The levels of C-reactive protein (CRP), tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) were detected by enzyme-linked immunosorbent assay (ELISA). All kits were purchased from Jingmei Biotech (Beijing, China) Co Ltd. Oxidative stress indicators: the level of malondialdehyde (MDA) was measured by thiobarbituric acid method. The kit was purchased from Jining Biotechnology Co., Ltd (Shanghai, China), and the level of superoxide dismutase -1(SOD-1) was detected by the radioimmunoassay. Angiogenesis-related factors: the levels of vascular endothelial growth factors (VEGF) and angiopoietin 2 (Ang-2) were detected by ELISA. VEGF kit was purchased from BD Biosciences (New Jersey, USA) and Ang- 2 kit was purchased from Xinbo Biotechnology Co., Ltd (Shenzhen, China).

Statistical methods

The data were processed using SPSS19.0 software; the measurement data were expressed by ($\bar{x}$ ± SD); the comparisons at different time were performed using measures analysis of variance, the intra-group comparison at two time points was performed using paired sample t test, the comparison at between groups was performed using independent sample t test. P<0.05 indicated that the difference was statistically significant.

Results

Completion status of the experiment

In this study, all 78 patients completed the entire experimental cycle, and effective indicators were obtained. The wounds in

all patients of the observation group were effectively closed through the experimentally designed regimen, the treatment course was 42 -71 days, with an average of (48.13 ± 3.37) days; the wounds in 5 patients in the control group were finally closed by surgical treatment when the wounds entered into red period, and the wounds in the remaining patients were closed by the experimentally designed regimen, the treatment course was 53-102 days, with an average of (64.72 ± 13.35) days.

Comparison of the changes in wound area between two groups

The wound changes in the time effect, grouping effect and interactive effect were statistically significant (F time point=130.288, F group=25.954, F time point × group=7.980; all P<0.001). The intra-group comparison showed that the wound areas after 2 weeks of treatment were significantly lower than those prior treated in the two groups, the differences were was statistically significant (P<0.05). The comparison at fixed time between groups showed that the wound areas in the observation group were significantly lower than those in the control group at the time points of 2 weeks, 3 weeks, 4 weeks and 5 weeks (Table 1).

Table 1. *Comparison of the change in wound area between two groups ($\bar{x}$ ± SD, cm^2).*

Group (n)	Before treatment	After 1 week of treatment	After 2 weeks of treatment	After 3 weeks of treatment	After 4 weeks of treatment	After 5 weeks of treatment
	8.94 ± 3.61	8.24 ± 2.46	6.21 ± 1.70[ab]	4.45 ± 0.84[abc]	3.01 ± 0.71[abcd]	1.22 ± 0.28[abcde]
Observation (39)	8.40 ± 3.13	7.67 ± 2.34	7.14 ± 1.06[a]	5.90 ± 1.08[abc]	5.03 ± 1.24[abcd]	3.34 ± 0.68[abcde]
T	0.707	1.042	2.932	6.601	8.819	17.977
P	0.482	0.301	0.000	0.000	0.000	0.000

Note: [a]compared with that before treatment, P<0.05; [b]compared with that after 1 week of treatment, P<0.05; [c]compared with that after 2 weeks of treatment, P<0.05; [d]compared with that after 3 weeks of treatment, P<0.05; [e]compared with that after 4 weeks of treatment, P<0.05.

Comparison of the levels of inflammatory factors before and after treatment between two groups

There were no significant differences in the levels of CRP, TNF-α and IL-6 before treatment between the two groups (P<0.05). the levels of the above mentioned indicators in the two groups were decreased after 30 days of treatment, and the intra-group differences were statistically significant (P<0.05); the levels of the above mentioned cytokines in the observation group were significantly lower than those in the control group after treatment (P<0.05), and the differences were statistically significant between the two groups (P<0.05) (Table 2).

Table 2. *Comparison of the levels of inflammatory factors before and after treatment between two groups ($\bar{x}$ ± SD).*

Group (n)	CRP(mg/L)		TNF-α(ng/ml)		IL-6(pg/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation (39)	4.31 ± 1.65	1.31 ± 0.42[a]	22.18 ± 3.04	13.74 ± 4.10[a]	67.58 ± 12.24	53.68 ± 8.28[a]
Control (39)	4.40 ± 1.28	2.84 ± 0.83[a]	21.58 ± 2.37	17.35 ± 3.58[a]	66.14 ± 14.25	59.85 ± 10.65[a]
t	0.269	10.272	0.972	4.142	0.479	2.586
P	0.789	0.000	0.334	0.000	0.634	0.006

Note: [a]compared with that before treatment, P<0.05.

Comparison of oxidative stress indexes before and after treatment between two groups

There were no significant difference in the levels of MDA and SOD-1 before treatment between the two groups (P>0.05). The levels of SOD-1 in the two groups were significantly decreased after 30 days of treatment, while the levels of MDA in the two groups SOD-1 were significantly increased after 30 days of treatment, and the intra-group differences were statistically significant (P<0.05). The level of MDA in the observation group was significantly lower than that in the control group, while the level of SOD-1 in the observation group was significantly higher than control group after treatment, and the differences were statistically significant between the two groups (P<0.05) (Table 3).

Table 3. *Comparison of oxidative stress indicators before and after treatment between two groups ($\bar{x}$ ± SD).*

Group (n)	MDA (μmol/L)	SOD-1 (μg/L)

	Before treatment	After treatment	Before treatment	After treatment
Observation (39)	4.95 ± 0.54	3.07 ± 0.35[a]	260.58 ± 33.84	328.71 ± 22.47[a]
Control (39)	4.77 ± 0.52	3.82 ± 0.42[a]	262.03 ± 32.45	298.53 ± 24.60[a]
t	1.499	8.567	0.188	5.657
P	0.138	0.000	0.852	0.000

Note: [a]compared with that before treatment, P<0.05.

Comparison of angiogenesis related indicators before and after treatment between two groups There were no significant differences in the levels of VEGF and Ang-2 before treatment between the two groups (P>0.05). The levels of above mentioned indicators in the two groups were increased after 30 days of treatment, and the intra-group differences were statistically significant (P<0.05). The levels of the above mentioned indicators in the observation group were significantly higher than those in the control group after treatment, and the differences were statistically significant between the two groups (P<0.05) (Table 4).

Table 4. *Comparison of angiogenesis related indicators before and after treatment between two groups ($\bar{x}$ ± s).*

Group (n)	VEGF (ng/L)		Ang-2	
	Before treatment	After treatment	Before treatment	After treatment
Observation (39)	287.41 ± 39.35	408.95 ± 39.12	23.19 ± 2.54	49.50 ± 2.92
Control (39)	293.18 ± 40.41	328.85 ± 48.58	23.56 ± 2.05	32.98 ± 3.30
t	0.641	8.020	0.708	23.413
P	0.523	0.000	0.481	0.000

Discussion

Our research found that Hyperbaric oxygen intervention can promote the healing of chronic wound in type 2 diabetes mellitus, which may be related to the inhibition of CRP, TNF-α, IL-6, MDA, and the promotion of SOD-1, VEGF and Ang-2. Diabetic chronic wound is a common refractory wound, its mechanism has not yet been clearly explained, but in which the nerve injury, microvascular disease, infection and oxidative stress reaction may play certain roles [13], and the active control of blood sugar and the establishments of effective blood supply and oxygen supply channels are the keys to promote wound healing. This study suggested that hyperbaric oxygen therapy can effectively promote chronic wound healing in diabetic patients: the patients in the observation group received hyperbaric oxygen therapy, and the wound area during the second to fifth week of treatment was significantly less than that in the control group, and the wounds were effectively closed after an average of (48.13 ± 3.37) days. Ueno et al. [14] reported 29 chronic wound patients, of whom, 13 patients with diabetes received 6 weeks of hyperbaric oxygen therapy, although 4 diabetic patients had poor wound healing, it was still indicated that the hyperbaric oxygen therapy had a certain value in promoting wound healing; Hongmei et al. [15] treated 44 diabetes patients with adjuvant hyperbaric oxygen therapy, the markedly effective rates was 77.3%, which was significantly better than 54.5% in the conventional treatment. The above-mentioned evidences suggest that hyperbaric oxygen has a better adjuvant treatment effect on chronic wounds in diabetic patients, and this study further explored its possible mechanisms.

The patients of both groups showed a strong inflammatory state in the early treatment, and the levels of CRP, TNF-α and IL-6 in the peripheral blood were relatively high. After 30 days of treatment, the above-mentioned indicators were significantly decreased along with the healing of the wound, indicating that the inflammation may be related to non-healing wound. The observation showed that the levels of acute phase protein and chemokine in blood circulation in most diabetes patients with foot ulcers were up regulated, this led to extensification of local infection and systemic inflammatory reaction, which would further cause abnormalities in the damage repair mechanism [16]. For instance, increased TNF-α level can lead to the persistence of wound inflammatory infiltration, and would inhibit the release of transforming growth factor-β1as well as inhibit the micro-angiogenesis [17]. In this study, the levels of CRP, TNF-α and IL-6 in the observation group were significantly lower than those in the control group after 30 days of treatment, which indicated that hyperbaric oxygen can effectively reduce inflammatory reaction, this is consistent with the results of the report on patients with severe craniocerebral injury [18]. This shows that decreasing the proinflammatory factor and inflammatory reaction may be one of the mechanisms for the hyperbaric oxygen to promote wound healing.

The MDA level was relatively high and SOD-1 level was relatively low in the two groups before treatment, the MDA level showed a decreasing trend and SOD-1 level showed an increasing trend during the treatment, suggesting that the oxidative stress reaction may also be related to the non-healing wound. At the hyperglycemia state, the glycosylation of antioxidant enzymes occurs, and the activities of antioxidant enzymes such as SDO and catalase are decreased and the reactive oxygen species are increased, which leads to increased production of intracellular reactive oxygen species, causes the cell toxicity and aggravated the blood vessel damage and axonal degeneration and neuropathic pain, and thus affecting the wound healing [19]. In this study, after 30 days of treatment, MDA level in the observation group was lower than that in the control group and SOD-1 in the observation group was higher than that in the control group, suggesting that hyperbaric oxygen therapy can reduce the body's oxidative stress status, which is not completely consistent with the report of Ma et al. [20]. The latter study showed that the hyperbaric oxygen therapy can not only increase the SOD-1 level, but also promote the MDA level, and indicated that the sustained hyperbaric oxygen therapy may exacerbate oxidative stress

reaction, the mechanism for the difference may be the fact that the patients with other types of chronic wounds were enrolled in the study in addition to patients with diabetic foots, and the disease condition may be different. The report of Yu et al. [21] on diabetic peripheral neuropathy indicated that the hyperbaric oxygen combined with α-lipoic acid can inhibit oxidative stress reaction, and the result was consistent with that of this study. And hyperbaric oxygen can improve the oxygen content of tissue, so that the wound environment is not conducive to the reproduction of anaerobic bacteria, while promoting the bactericidal effect of white blood cells, improve phagocytosis of macrophages. Hyperbaric oxygen promotes the oxygen dependent peroxidase system in leukocytes, increases the production of oxygen free radicals, enhances the oxidation of proteins and membrane lipids, and inhibits bacterial metabolism [22]. Hyperbaric oxygen can also reduce proinflammatory factors and reduce inflammatory response [23]. Hyperbaric oxygen improves the local blood flow, improves the blood concentration of antibiotics in tissues, and enhances the ability of certain antibiotics to cross the bacterial cell wall through oxygen transport [24].

In this study, we found that VEGF and Ang-2 levels in the observation group were significantly higher than those in the control group after 30 days of treatment, suggesting that hyperbaric oxygen can promote angiogenesis, this is consistent with the result of the other report [25]. The increased oxygen supply may be conducive to improve local cell metabolism and promote cell function recovery, thereby accelerating fibroblast division and promoting microvascular repair and capillary angiogenesis. This helps to improve microcirculation and promote wound healing [26,27]. In summary, hyperbaric oxygen therapy can promote the healing of chronic wounds in patients with type 2 diabetes, which may be related to its abilities to inhibit the inflammatory reaction and oxidative stress reaction and promote the angiogenesis.

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