# Change of cytokine in pressure ulcer with undermining

**Original Article**

Journal of the Tsuruma HEALTH SCI. MED. KANAZAWA UNIV. Vol. 28
25～30 2004

# Change of cytokine in pressure ulcer with undermining

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## ABSTRACT

The purpose of this study was investigated the change in cytokines in pressure ulcers with undermining and relationship between its changing and wound healing process. We investigated cytokines in pressure ulcers with undermining and wound change patterns in order to clarify cytokine behaviour at each status. Monthly measurements were performed of the levels in retained exudate of the cytokines; interleukin 1$\alpha$ and 1$\beta$ (IL-1$\alpha$, 1$\beta$), basic fibroblast growth factor (bFGF), platelet-derived growth factor-AB (PDGF-AB), interleukin-4 (IL-4), transforming growth factor-$\beta$1 (TGF-$\beta$1) and vascular endothelial growth factor (VEGF). Wounds were observed weekly. All subjects had consented to participate in the study. Cytokine measurements were performed at six wound sites. Only IL-1$\alpha$, 1$\beta$ and VEGF were found in detectable quantities in all exudate samples; all of the others were undetectable at various times. Pressure ulcers with delayed healing at the time of the study had lower rates of detection of IL-4 and bFGF compared with other pressure ulcers. Cytokines were increased at each wound change: for undermining adhesion, it was five out of seven (elevation in VEGF); for wound contraction, three out of four (bFGF); for increased granulation on the side of the undermining, three out of three (bFGF); and for epithelialization also three out of three (bFGF). Therefore, it was suggested that VEGF and bFGF were related to the promotion of healing in the pressure ulcers with undermining.

## KEY WORDS

cytokine, pressure ulcer, undermining, healing process

## Introduction

Pressure ulcers with undermining are refractory because they take longer to heal than pressure ulcers without undermining[1]. Although elderly patients are more likely to have pressure ulcers with undermining, pressure ulcer care guidelines do not include nursing procedures for pressure ulcers with undermining[2-5].

Previous research has identified three distinctive characteristics of the healing process in pressure ulcers with undermining[6] : 1) Even with the proliferation of granulation tissue on the side of the undermining, there is no proliferation of granulation tissue in the wound bed, and it is difficult for granulation tissue to adhere to the undermining surface 2) Epithelialization occurs, leaving the undermining and the wound closes up. 3) Even if granulatior tissue adheres to the undermining surface, epithelia lization does not occur at the site of a previou undermining. Further investigation revealed the cause of these characteristics to be: pressure, shear an bacterial contamination of the wound, caused b external bony prominence, contracture, loose skin i the buttock area, and wound contamination throug incontinence. Pressure causes tissue damage by reduc ing or cutting off the blood flow, restricting th supply of oxygen and nutrients to the wound are

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and encouraging overproduction of active oxygen. Shear causes mechanical damage to granulation tissue in the undermining and wound bed, while inflammation associated with wound contamination due to incontinence delays wound healing. The presence of inflammation suggests that a different wound healing system applies in pressure ulcers with undermining compared with the normal healing process for pressure ulcers without undermining. Previous studies relating to pressure ulcers with undermining have been reported in terms of patient survival rates[7], healing times[1], and the factors that lead to undermining[3,8] ; however, there has been no attempt to investigate the associated wound healing system. Therefore, we focused on cytokine matrices that are used as indicators of the healing process from the perspective of molecular biology. In this study, we analysed the relation between the change of cytokine levels and the healing process in pressure ulcers with undermining.

## Methods

Cytokine levels in pressure ulcers with undermining were analysed alongside changes in the wound in order to identify the characteristics of cytokine behaviour at each status.

### 1. Sample

Subjects were residents of long-term care hospitals who had pressure ulcers with undermining.

### 2. Survey items

1) Cytokines

Monthly measurements were performed of levels in the retained exudate of interleukin-1$\alpha$ (IL-1$\alpha$), interleukin-1$\beta$ (IL-1$\beta$), basic fibroblast growth factor (bFGF), platelet-derived growth factor-AB (PDGF-AB), interleukin-4 (IL-4), transforming growth factor-$\beta$1 (TGF-$\beta$1) and vascular endothelial growth factor (VEGF). Cytokines were measured by ELISA (enzyme-linked immunosorbent assay). Exudate samples were stored at $-70℃$ until assayed.

2) Wound changes

Photographs of the wounds were taken every week and macroscopic findings were recorded. Wounds were also assessed weekly using the DESIGN scale[9]. The wound condition at the time of cytokine sampling was compared with the condition that was observed in the previous week, and the differences were defined as the wound changes.

### 3. Exudate sampling

Pressure ulcer exudate was sampled using the following non-invasive technique:

1) The wound and surrounding area was washed and then covered with a transparent dressing.
2) After 2-3 h, an exudate sample was taken from the wound using a cotton bud and the sample placed in a test tube.
3) The weight of the exudate sample was measured in grams using electronic scales, with precision to three decimal places.
4) Normal saline (1 mL) was added to the test tube containing the cotton bud.
5) The test tube was agitated for 10 s three times in a vortex mixer at a speed of 2800 rpm.
6) The cotton bud was removed from the test tube and the diluted exudate analysed for the presence of cytokines.

The analysis results were multiplied by the dilution ratio. For example, if 0.050 g of exudate sample was diluted with 1 mL of normal saline, the analysis results were multiplied by a factor of 20. This sampling method has been validated previously using isotopes.

### 4. Analysis

Cytokine levels at each status were classified as to whether healing was delayed or in progress at the time of the first observation, and detection rates for the different types of cytokines were then compared. The wound change of each pressure ulcer with undermining was shown by the frequency. We compared the rate of increase in the levels of each type of cytokine for each wound change in each phase.

### 5. Ethical considerations

Prior to the present study, the hospital director and nursing manager were briefed on the nature of this study, and their consent was obtained. Patients and their families were also briefed on the study and their consent was also obtained. Patients and their families were informed that refusal to participate in the present study would not affect their future treatment or care in any way, and that they had the right to withdraw from the study at any time.

Table 1. Subject demographics (n=6)

| Subject | Age (yr) | Gender | Diagnosis | Pressure ulcer site | Depth | Period of delay in healing at the time of the first observation (mth) | Stage during survey | Study duration (mth) | Pressure ulcer present (mth) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 75 | F | Renal impairment | sacral | Ⅳ | 2 | proliferation | 7 | 24 |
| 2 | 93 | F | Heart failure | sacral | Ⅲ | 2 | proliferation | 6 | 24 |
| 3 | 74 | F | Post-stroke | PSIS* | Ⅳ | 1 | proliferation | 4 | 6 |
| 4 | 86 | M | Dementia | sacral | Ⅳ | 0 | proliferation | 2 | 4 |
| 5 | 91 | F | Myocardial infarction | PSIS* | Ⅲ | 0 | proliferation | 2 | 1 |
| 6 | 75 | F | Stroke, pneumonia | sacral | Ⅳ | 0 | inflammatory | 3 | 0.25 |

*PSIS: posterior superior iliac spine

**Results**

**1. Subjects** (Table 1)

The study sample consisted of six subjects with six pressure ulcers with undermining. Subject ages ranged from 74 to 93 years. There was one male and five female subjects. Two subjects suffered from cardiac failure, three from cerebrovascular disease and one from kidney disease. Four pressure ulcers were located in the sacral area, and the other two over the posterior superior iliac spine. Four ulcers were stage IV and two were stage III according to the NPUAP classification system. Five of the ulcers were in the proliferation phase at the time of the present study, while the remaining ulcer was in the inflammatory phase. Half of the ulcers exhibited delayed healing at the time of the first observation (Subject No. 1, 2, 3), with ulcers present for between 0 and 24 months.

**2. Cytokine detection**

A total of 24 measurements were performed. Only IL-1$\alpha$, 1$\beta$ and VEGF were found in detectable quantities in all exudate samples; the others were undetectable at various times.

**3. Relation between cytokines and wound changes at each status**

Cytokine detection rates (excluding IL-1$\alpha$, 1$\beta$ and VEGF, which were detected throughout) were analysed according to whether healing was in progress at the time of the test. Of the 17 tests of the wounds conducted delayed healing at the time of the first observation, TGF-$\beta$1 was detected nine times (52.9%), bFGF seven (41.2%), PDGF-AB four (23.5%) and IL-4 three (17.6%). Out of the seven tests of the wounds considered healing in progress bFGF was detected six times (85.7%), TGF-$\beta$1 five times (71.4%), IL-4 four times (57.1%) and PDGF-AB once (14.3%) (Table 2).

Increases in cytokine levels were then analysed for each wound change in each phase. Wound changes in the proliferation phase included undermining adhesion (seven times), wound contraction (four times), increase in granulation tissue on the side of the undermining (three times), and epithelialization (three times). The only wound change observed in the inflammatory phase was increased granulation in the wound bed (once). There were some instances of delayed healing during the study period, but no instances of the wound condition deteriorating. Cytokines were increased at each wound change during the proliferation period: for undermining adhesion it was five out of seven (VEGF, 71.4%); for wound

Table 2. Wound status at the first observation and cytokine detection times

| Wound status | Times | IL-1α | IL-1β | bFGF | PDGF-AB | IL-4 | TGF-β1 | VEGF |
|---|---|---|---|---|---|---|---|---|
| Delayed healing | 17 | 17 | 17 | 7 | 4 | 3 | 9 | 17 |
| Healing | 7 | 7 | 7 | 6 | 1 | 4 | 5 | 7 |

Table 3. Wound changes and increase in cytokines

| Wound Changes | Times | IL-1α | IL-1β | bFGF | PDGF-AB | IL-4 | TGF-β1 | VEGF |
|---|---|---|---|---|---|---|---|---|
| Undermining adhesion | 7 | 3 | 4 | 2 | 3 | 2 | 3 | 5 |
| Wound contraction | 4 | 2 | 2 | 3 | 0 | 2 | 1 | 0 |
| Proliferation of granulation tissue in undermining | 3 | 1 | 0 | 3 | 0 | 2 | 1 | 0 |
| Epithelialization | 3 | 1 | 2 | 3 | 0 | 0 | 1 | 2 |

contraction, three out of four (bFGF, 75.0%); for increased granulation tissue on the side of the undermining, three out of three (bFGF, 100.0%); and for epithelialization, again three out of three (bFGF, 100.0%) (Table 3).

## Discussion

It has been reported that cytokine concentrations tend to be lower in pressure ulcers than in ordinary wounds[10]. Previous studies of pressure ulcers have measured cytokine levels at specific times, but have not attempted to track chronological changes in cytokine levels. Growth factors are known promoters of wound healing. Many studies in recent years have reported that cytokines are also beneficial for the healing of pressure ulcers[11-12], but there have been no reports on the benefits for pressure ulcers with undermining. There is no concrete information about the promotion of healing of pressure ulcers with undermining, and so no systematic guidelines exist. This study investigated the correlation between changes in cytokine levels and the healing process in pressure ulcers with undermining. Pressure ulcers with undermining are particularly susceptible to delayed healing and are unsuitable for in vitro experimentation, and no animal models have been developed to investigate undermining and undermining formation in pressure ulcers. In this sense, the cytokine test results for pressure ulcers with undermining in human subjects are highly significant. The present study provides extremely valuable information for use in the development of patient care regimes and techniques for promoting the healing process of pressure ulcers with undermining.

Hereafter, the effect of cytokines on the healing process in pressure ulcers with undermining is considered.

### 1. Characteristics of cytokines in pressure ulcers with undermining

IL-1$\alpha$, 1$\beta$ and VEGF were found in detectable quantities in all exudate samples; the other cytokines

were undetectable in some samples. IL-1$\alpha$, 1$\beta$, a pro-inflammatory cytokine, is produced by monocytes, macrophages and vascular endothelial cells in response to stimulation by bacteria and associated endotoxins during the highly acute inflammation phase[13] Thus, the causes of inflammation are constantly at play in pressure ulcers with undermining, extending the inflammatory reaction. Because hypoxia and low glucose levels boost the production of VEGF[14], the blood flow in pressure ulcers is unlikely to improve. It is noteworthy that cytokines bFGF, TGF-$\beta$1 and PDGF-AB, which promote wound healing, were not detected in all tests.

From the molecular biology perspective, although undermining formation makes it more difficult for pressure ulcers to heal, proper patient care to reduce pressure, and eliminate shear and wound contamination for shortening of the inflammatory reaction and promotion of the blood flow helps to promote the healing process.

**2. Effect of cytokines on the healing process of pressure ulcers with undermining**

Both IL-4 and bFGF levels during the survey period were lower in pressure ulcers with periods of delayed healing than in progressive healing at the time of the first observation. IL-4 is an anti-inflammatory cytokine, which in the present study inhibited production of the pro-inflammatory cytokine, IL-1$\beta$. Delayed healing in pressure ulcers with undermining can therefore be attributed to marked inflammation. Another contributing factor would be the low detected levels of the cytokine, bFGF, which has a significant positive effect on wound healing.

By wound change and phase, VEGF had a higher rate of increase for undermining adhesion, while bFGF had a higher rate of increase for wound contraction, increased granulation tissue on the side of the undermining, and epithelialization. VEGF stimulates neovascularisation, but in addition to boosting the propagation of endothelial cells, it also promotes luminal formation and the resolution of extracellular matrices as required for angiogenesis[15]. The cytokine VEGF is therefore thought to be involved in the adhesion of undermining granulation tissue on the side of the undermining to granulation tissue on the side of the wound bed. bFGF, meanwhile, increases neovascularisation and fibroblast propagation and promotes epithelialization[16], and is thought to be involved with the healing processes for wound contraction, granulation on the side of the undermining and epithelialization. The fact that the levels of bFGF and VEGF were higher during the healing process of pressure ulcers with undermining suggests that, conversely, healing will be delayed when the bFGF and VEGF values are low. The bFGF preparations currently licensed for use in Japan as growth factor should provide an effective means for countering delayed healing in pressure ulcers with undermining.

Higher levels of the pro-inflammatory cytokine, IL-1$\beta$, were detected in pressure ulcers with undermining, and lower levels of the anti-inflammatory cytokine, IL-4, in ulcers with delayed healing. From this we can conclude that inflammation obstructs the healing process in pressure ulcers with undermining.

One of the limitations of the present study was that the samples were only pressure ulcers with undermining. To investigate the effect of cytokines on the healing process of pressure ulcers with undermining at the molecular biology level, it will be necessary to compare cytokine levels in pressure ulcers with undermining to those in pressure ulcers without undermining.

**Conclusions**

The only cytokines that were detected consistently in all tests on exudate taken from pressure ulcers with undermining were IL-1$\alpha$, 1$\beta$ and VEGF, indicators of the inflammatory reaction. Cytokines that promote wound healing, bFGF, TGF-$\beta$1 and PDGF-AB were not detected in wound exudates at every status. The detection rates of IL-4 and bFGF were clearly lower in pressure wounds with delayed healing. Both VEGF and bFGF levels increased sharply in pressure ulcers with undermining when healing was advanced. We consider that these results indicate the impact of inflammation in delaying the healing process of pressure ulcers with undermining. Thus, it should be possible to promote healing by preventing damage through basic patient care techniques to reduce pressure, shear and wound contamination, and also by administering bFGF preparations.

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# ポケットを有する褥瘡のサイトカインの推移

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要　　旨

本研究の目的は，ポケットを有する褥瘡のサイトカインの推移と治癒過程の関係を検討することである。方法は，ポケットを有する褥瘡のサイトカインと創の変化を調査し，創の病期別にサイトカインの推移の特徴を導いた。サイトカインは貯留した浸出液から IL-1α・1β，bFGF，IL-4，TGF-β1，VEGF，PDGF-AB を１ヶ月毎に測定し，創は1週間ごと観察した。なお，対象者からは研究協力の承諾を得た。その結果，６部位の褥瘡から，サイトカインを24回調査した。浸出液中からは毎回定量可能なレベルで IL-1α・1β，VEGF が検出されたが，その他は毎回検出されないこともあった。調査時に治癒停滞しているか否かで分類して比較すると，治癒停滞の認めた褥瘡は認めない褥瘡より IL-4 と bFGF の検出率は低くかった。創の変化別にサイトカインの上昇した割合（上昇回数/創の変化回数）をみると，ポケットの接着は VEGF が5/7，創収縮は bFGF が3/4，ポケット皮膚側の肉芽増殖は bFGF が3/3，表皮化は bFGF が3/3と高率であった。以上より，ポケットを有する褥瘡の治癒には VEGF と bFGF が関与していると示唆された。