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Original Article

Pathological characteristics and cytokine and growth factor profiles of pressure ulcers: a prospective single center study

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Abstract: Objective: We investigated the pathologic characteristics of National Pressure Ulcer Advisory Panel (NPUAP) grade 3 and 4 pressure ulcers and further elucidated the profile of inflammatory cytokines and growth factors in pressure ulcer tissues. Methods: Pressure ulcer tissues specimens were obtained from 66 patients with 82 pressure ulcers and normal skin tissue specimens from 20 healthy subjects. The contents of EGF, TGF-β, β-FGF, PDGF-BB, and VEGF in the tissue samples were examined by ELISA. Results: Pathological examination revealed that pressure ulcers could be categorized into degenerative (n=16), effusive (n=9) and proliferative pressure ulcer (n=7). The baseline levels of EGF and VEGF were significantly lower than those of normal controls (P<0.05). By contrast, the baseline levels of TGF, β-FGF and PDGF-BB were significantly elevated compared with those of normal controls (P<0.01 or 0.05) while no statistically significant difference was observed in the baseline levels of IL-8 of pressure ulcer patients and controls (P>0.05). The levels of these cytokines and growth factors also varied by pathologic types. Furthermore, surgical treatment impacted on the levels of these factors. Conclusions: Pressure ulcers can be categorized into distinct pathologic types and these different types of pressure ulcers are associated with distinct patterns of changes in cytokines and growth factors.

Keywords: Epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, pressure ulcers, transforming growth factor-β, vascular endothelial growth factor

Introduction

Pressure or decubitus ulcer is a localized injury to the skin and/or underlying tissue as a result of pressure, or pressure in combination with shear and/or friction, typically over a bony prominence. It increases the risk for infection, delays patient recovery, and may lead to mortality in certain patients [1, 2]. It also poses a significant financial burden on patients, family and healthcare systems worldwide. Clinically, the severity of pressure ulcer is determined by the depth of an ulcer while its pathological stage is not considered. The wound surface of an ulcer is often protracted; enlarged or complicated by infection, and progressive necrosis of the skin flap may even occur [3-8]. Preoperative evaluation, hydrocolloid or foam dressings and protein or amino acid supplementation are recommended pressure ulcer patients [9].

The mechanisms for the pathogenesis of pressure ulcers remain largely unelucidated. Pressure ulcer healing not only requires the coordinated efforts of multiple cell types but also involves a complex network of cytokines and growth factors [10]. Hitherto, few studies have delineated the profile of cytokines and growth factors in pressure ulcer tissues. Jiang *et al.* demonstrated that pressure ulcer tissues exhibited markedly increased mRNA transcript levels of inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) and significantly lower mRNA transcript levels of vascular endothelial growth factor (VEGF) and β-platelet-derived growth factor (β-FGF) compared with controls [11]. A recent meta analysis showed that topical recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF) may have a positive effect on pressure ulcers [12].

Table 1. Demographic and baseline characteristics of the study patients

	All patients	Degenerative pressure ulcer	Effusive pressure ulcer	Proliferative pressure ulcer	*P*
N	66	16	9	7	
Age, years	20-79	32-65	26-70	20-79	
Gender					
Male	46	12	7	6	
Female	26	4	2	1	
BMI, kg/cm^2					
Location	82	23	20	15	
Sacrococcygeal	50	15	12	3	
Greater trochanter of the femur	20	6	6	5	
Ischial tuberosity	12	2	2	7	
Braden Scale score	10	9	10	11	

In the current study, we investigated the pathologic characteristics of National Pressure Ulcer Advisory Panel (NPUAP) grade 3 and 4 pressure ulcers and further elucidated the profile of inflammatory cytokines and growth factors in pressure ulcer tissues.

Patients and methods

Patients

Patients with pressure ulcers who sought treatment at our department between September 2008 and June 2013 were recruited. Patients with grade 3 or 4 pressure ulcers according to the NPUAP staging criteria were included in the study. Patients who had incomplete preoperative or postoperative specimens or specimens from normal skin sites were excluded. Surgical specimens from ulcer tissues of pressure ulcer patients and from normal skin tissues of 20 healthy subjects were obtained.

The study protocol was approved by the local institutional review board at the First Affiliated Hospital of Jilin University and written patient consent was obtained all study patients or their legal surrogates.

ELISA

The contents of IL-8, EGF, TGF-β, β-FGF, PDGF-BB, VEGF were determined using commercially available ELISA kits as instructed by the manufacturer. The OD for IL-8 and β-FGF was read at 610-630 nm and for EGF, PDGF-BB, VEGF and TGF-β at 450 nm.

Statistical analysis

Data were expressed as $\bar{x}\pm s.d.$ and analyzed using SPSS version 17.0. Student's t test was used for comparison of differences between groups. *P* values <0.05 were considered statistically significant.

Results

Demographic and baseline characteristics of the study patients

Sixty-six patients with a total of 82 pressure ulcers received treatment at our institution during the review period. The demographic and baseline characteristics of the study patients are shown in **Table 1**. They included 46 (69.7%) males and 20 (30.3%) females aged 20- to 9 years. They included 50 (61.0%) sacrococcygeal pressure ulcers, 20 (24.4%) pressure ulcers of the greater trochanter of the femur, and 12 (14.6%) ischial tuberosity pressure ulcers. The formation time of pressure ulcers ranged from 1 month to 28 years. The extent of pressure ulcers ranged between 10 cm×20 cm and 20 cm×40 cm. Their mean Braden Scale score was 13.6±0.5 (range 2 to 20).

Pathological and surgical characteristics of the study patients

Based on gross and pathological examination, 40 (48.8%) pressure ulcers were categorized into degenerative pressure ulcer, which was characterized by degenerative inflammation, with mild effusion and scant proliferation (**Figure 1A**), 28 (34.1%) into effusive pressure

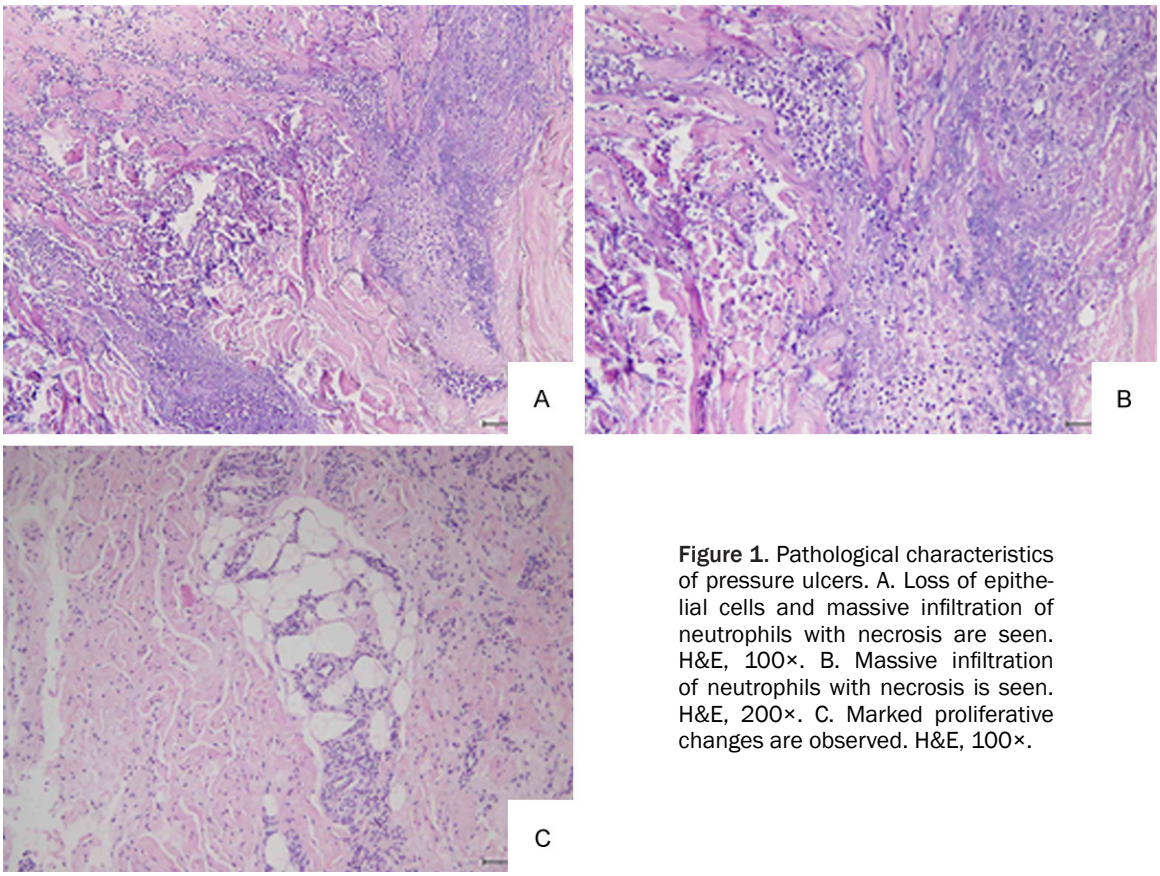

Figure 1. Pathological characteristics of pressure ulcers. A. Loss of epithelial cells and massive infiltration of neutrophils with necrosis are seen. H&E, 100×. B. Massive infiltration of neutrophils with necrosis is seen. H&E, 200×. C. Marked proliferative changes are observed. H&E, 100×.

ulcer, which was characterized by serous inflammation with effusion of non-viscous serous fluid, fibrinogen and neutrophils (**Figure 1B**) and 14 (17.1%) into proliferative pressure ulcer, which was characterized by chronic inflammation marked by proliferative changes (**Figure 1C**).

For degenerative pressure ulcers, 19 were managed with split thickness skin graft. In addition, 54 ulcers were managed with local skin flaps (n=21), pedicled muscle flaps (n=27) or free flaps (n=6). Nine ulcers were directly sutured. For effusive pressure ulcers, skin graft surgery was performed in 6 ulcers and flap repair in 22 ulcers. Proliferative ulcers received phase I flap repair. The cure rate was 90% for degenerative pressure ulcers, 92.9% for effusive pressure ulcers, 92.8% for proliferative pressure ulcers.

Cytokine and growth factor profiles of the study patients

We examined the levels of cytokines and growth factors in the pressure ulcer tissues by ELISA. We found that the baseline levels of EGF (6.2±1.9 pg/mL) and VEGF (62.9±21.2 pg/mL) were significantly lower than those of normal controls (EGF: 13.7±2.2 pg/mL; VEGF: 155.5±30.9 pg/mL) ($P<0.05$). By contrast, the baseline levels of TGF (45.8±10.3 pg/mL), β-FGF (1076.9±131.1 pg/mL) and PDGF-BB (3240.7±211.2 pg/mL) were significantly elevated compared with those of normal controls (TGF: 37.7±4.8 pg/mL; β-FGF: 90.6±10.7 pg/mL; PDGF-BB: 998.4±145.9 pg/mL) ($P<0.01$ or 0.05) while no statistically significant difference was observed in the baseline levels of IL-8 of pressure ulcer patients (5570.2±124.7 pg/mL) and controls (5223.4±167.9 pg/mL) ($P>0.05$). Surgical therapy significantly increased the levels of EGF (6.2±1.9 pg/mL; $P<0.05$ vs. baseline) and VEGF (62.9±21.2 pg/mL; $P<0.05$ vs. baseline), which, however, were still significantly lower than those of the controls ($P<0.05$). On the other hand, surgical treatment markedly reduced the levels of TGF (45.8±10.3 pg/mL; $P<0.05$ vs. baseline), β-FGF (1076.9±131.1 pg/mL; $P<0.05$ vs. baseline) and PDGF-BB (3240.7±211.2 pg/mL; $P<0.05$ vs. baseline).

Table 2. Cytokine and growth factor levels of the study patients

	Degenerative pressure ulcer	Effusive pressure ulcer	Proliferative pressure ulcer	*P*
N	16	9	7	
IL-8, pg/mL				
Preoperative	5570.2±124.7	5454.2±142.3	5420.2±118.9	
Postoperative	5446.1±213.8	5232.1±113.9	5416.1±163.8	
EGF, pg/mL				
Preoperative	6.2±1.9	11.5±2.1	7.1±2.8	
Postoperative	8.5±1.4	14.9±3.4	8.8±1.8	
TGF, pg/mL				
Preoperative	45.8±10.3	84.3±11.6	15.6±3.3	
Postoperative	16.5±2.4	15.6±3.1	32.1±8.9	
β-FGF, pg/mL				
Preoperative	1076.9±131.1	1432.2±124.6	136.9±37.1	
Postoperative	145.7±14.6	126.5±12.8	102.4±11.8	
PDGF-BB, pg/mL				
Preoperative	3240.7±211.2	1736.1±145.9	1234.5±134.2	
Postoperative	1285.6±198.3	937.5±98.4	1012.1±98.7	
VEGF, pg/mL				
Preoperative	62.9±21.2	108.8±24.9	98.9±11.8	
Postoperative	98.9±18.7	90.8±12.1	146.1±11.8	

The normal control values at our hospital: IL-8: 5223.4±167.9 pg/mL; EGF: 13.7±2.2 pg/mL; TGF: 37.7±4.8 pg/mL; β-FGF: 90.6±10.7 pg/mL; PDGF-BB: 998.4±145.9 pg/mL; VEGF: 155.5±30.9 pg/mL.

Cytokine and growth factor profiles of the study patients according to the pathological types of pressure ulcers

We further analyzed the levels of cytokines and growth factors in the pressure ulcer tissues according to the pathological types of pressure ulcers. Patients with degenerative, effusive and proliferative pressure ulcers all showed significantly lower baseline levels of VEGF than normal controls ($P<0.01$ or 0.05) (**Table 2**). Furthermore, the baseline levels of VEGF in degenerative pressure ulcer tissues (62.9±21.2 pg/mL) were significantly lower than those of effusive (108.8±24.9 pg/mL) and proliferative pressure ulcers (98.9±11.8 pg/mL) ($P<0.05$). The postoperative levels of VEGF in degenerative (98.9±18.7 pg/mL) and effusive pressure ulcer tissues (90.8±12.1 pg/mL) still remained significantly depressed compared to controls ($P<0.05$). Only proliferative pressure ulcer tissues showed postoperative VEGF levels (146.1±11.8 pg/mL) comparable to controls ($P>0.05$). Patients with degenerative and proliferative pressure ulcers exhibited markedly lower baseline levels of EGF compared to controls ($P<0.05$) while effusive pressure ulcer tissues showed similar baseline EGF levels to those of normal controls ($P>0.05$). Surgical therapy resulted in no significant increase in EGF levels in degenerative and proliferative pressure ulcer tissues ($P>0.05$ vs. baseline and controls).

Though the baseline levels of TGF were significantly elevated for the study population compared with those of normal controls, they were markedly lower in proliferative pressure ulcer tissues (15.6±3.3 pg/mL $P<0.05$ vs. controls and degenerative pressure ulcer: 45.8±10.3 pg/mL and pressure effusive ulcer: 84.3±11.6 pg/mL) while they were comparable in degenerative pressure ulcer tissues and controls ($P>0.05$). Furthermore, surgical therapy significantly reduced the levels of TGF in degenerative and effusive pressure ulcers ($P<0.05$). We also observed significantly higher baseline levels of β-FGF in degenerative and effusive pressure ulcer tissues compared to controls ($P<0.01$), which, however, were marked reduced as a result of surgical therapy ($P>0.05$ vs. controls). In addition, degenerative pressure ulcer tissues exhibited the highest baseline levels of PDGF-BB (3240.7±211.2 pg/mL). Effusive pressure ulcer tissues also showed markedly higher baseline levels (1736.1±145.9 pg/mL) of PDGF-BB than controls ($P<0.05$) while proliferative pressure ulcer tissues had comparable levels to controls ($P>0.05$).

Discussion

Pressure ulcer poses a significant challenge clinically and in terms of healthcare cost worldwide. Pressure ulcer healing is a complicated dynamic process involving multiple cell types and cytokines and growth factors [10]. The protracted nature of pressure ulcer healing has been reported to be associated with reduction in the amount of growth factors and increases in the levels of proinflammatory cytokines [10,

13, 14]. The current study demonstrated that the levels of EGF and VEGF in pressure ulcer tissues were significantly lower than normal skin tissues. By contrast, we observed markedly higher levels of TFG, β-FGF and PDGF-BB in pressure ulcer tissues compared with controls. These findings indicate that growth factors exhibit distinct patterns of changes. Furthermore, we found that although pressure ulcer patients had markedly lower levels of EGF, patients with effusive pressure ulcer had comparable levels of EGF to controls. Similarly, higher levels of PDGF-BB were observed in pressure ulcer tissues for the study population, but proliferative pressure ulcer tissues expressed similar levels of PDGF-BB to the controls. The findings suggest that changes in cytokines or growth factors in pressure ulcer tissues may vary with the pathological types of pressure ulcers.

Ford *et al.* [15] and McGrath *et al.* [16] showed that EGF, FGF, and PDGF promoted formation of granulation tissue. Fu *et al.* [17] showed that β-FGF promoted recovery of burns and other protracted wounds. It has been demonstrated that β-FGF acts on its target cells (fibroblasts and vascular endothelial cells) to promote their proliferation [18, 19]. B-FGF also shortened hospital stay and reduced healthcare cost [20]. Consistently, our study also showed that, compared to controls, β-FGF levels increased more than 10 folds in degenerative and effusive pressure ulcers, suggesting intense ongoing wound healing activity in pressure ulcer tissues. Interestingly, surgical treatments reduced β-FGF levels to the control levels. Similar findings were observed in the postoperative levels of PDGF-BB. Lu *et al.* [21] demonstrated that the levels of EGF, FGF, and PDGF in burn tissues of patients with second degree burns who did receive surgical treatment were lower than those who received surgical treatment and granulation and epithelialization were impaired in the patients with second degree burns who did receive surgical treatment. This is consistent with our findings. Iocono *et al.* [22] found increased IL-8 levels in the wound recovery phase, which returned to normal levels after wound healing had completed. However, we failed to demonstrate significant increases in IL-8 levels in pressure ulcer tissues regardless of the pathological type.

The current study is limited by the small number of subjects included in the analysis and its single center nature. Prospective, multicenter studies involving a larger population size are needed in the future. In conclusion, pressure ulcers can be categorized into distinct pathologic types and these different types of pressure ulcers are associated with distinct patterns of changes in cytokines and growth factors.

Disclosure of conflict of interest

None.

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