Simple determination of basic fibroblast growth factor (bFGF) levels in the wound fluids of elderly patients with pressure ulcers; a significant correlation between the bFGF level and the DESIGN score

TADAKAZU KUBO[1], MAYURI TANAKA[2], YOSHIHIRO OKAMOTO[2]*, ATSUSHI TANAKA[3], REIKO SEKI[3], MASACHIKA TSUJI[1], TAKASHI FUKUI[2], TOSHIYUKI MASUZAWA[2]

[1] *Ebihara Hospital, 630-10, Futomi, Kamogawa, Chiba 299-2862, Japan*
[2] *Laboratory of Immunology and Microbiology, Faculty of Pharmacy, Chiba Institute of Science, 3 Shiomi-cho, Choshi, Chiba 288-0025, Japan*
[3] *Department of Dermatology, Kameda Medical Center, 929 Higashi-cho, Kamogawa, Chiba, 296-8602, Japan*

**Correspondence: Yoshihiro Okamoto*
Faculty of Pharmacy, Chiba Institute of Science, 3 Shiomi-cho, Choshi, Chiba 288-0025, Japan.
TEL +81 479 30 4686; FAX +81 479 30 4501; email: yokamoto@cis.ac.jp

ABSTRACT

The prevention of malignant outcomes from pressure ulcers is an ongoing process. Pharmacists participate in the assessment and treatment of pressure ulcers in the wards and advise medical staff on pharmacological effects, dosage, and adverse effects. The work includes monitoring malignancy and managing drugs and dressings. The assessment of basic fibroblast growth factor (bFGF) levels in wound fluids provides useful information for the healing of pressure ulcers. The aim of this study was to determine the bFGF level in wound fluids of patients with pressure ulcers. Sterile gauze was placed over the wound for 1 minute, and then it was soaked in phosphate buffer. This method is simpler than previous procedures and is also less stressful for patients. There was a significant negative statistical correlation (bFGF *vs* DESIGN total score: p= 0.0351, Spearman r=-0.483). This study demonstrated that this novel method for the bFGF determination of wound fluids was useful for the assessment of clinical severity.

Keywords: Basic fibroblast growth factor (bFGF), FGF2, Pressure ulcer, Wound fluid, DESIGN tool

INTRODUCTION

The complex and multiple processes leading to wound healing are controlled by cytokines and growth factors within the wound (Cooper *et al.*, 1994; Vogt *et al.*, 1998). Numerous studies have speculated that further assessment of these factors may yield important clues regarding failed wound healing and indeed may indicate specific growth deficiencies (Grose *et al.*, 2003; Li *et al.*, 2003; Werner *et al.*, 2003).

Basic fibroblast growth factor (bFGF), which is also called FGF2, is one of the most powerful angiogenic peptides (Bikfalvi *et al.*, 1997). It has pleiotropic effects when applied to various tissues, including the mitogenesis and differentiation of endothelial cells and fibroblasts and the stimulation of fibroblasts to produce collagen, and it is an important and early link in the wound healing cytokine cascades (Thomas, 1987; Greenhalgh *et al.*, 1990; Gallicchio *et al.*, 1991; Mayahara *et al.*, 1993; McLeskey *et al.*, 1994).

The secretion of bFGF in damaged tissues is significantly impaired, and local high concentrations of fibroblast growth factor promote granulation tissue formation and re-epithelisation (Fu *et al.*, 1996a; Fu *et al.*, 1996b). In impaired wounds such as foot ulcers, a local deficiency in bFGF may lead to delayed wound healing (Fu *et al.*, 1998).

Various measures of wound healing have been reported and applied in clinical assessment and research studies. However, a more objective and easy tool to track pressure ulcers is required. Therefore, the assessment of bFGF levels in wound fluids would provide useful information about the healing of pressure ulcers. The standard procedure for the collection of the wound fluids from patients is quite complicated. Furthermore, it is stressful for patients to undergo wound fluid collection (Cooper *et al.*, 1994, Vogt *et al.*, 1998; Trengove *et al.*, 2000; Gohel *et al.*, 2008).

As members of the medical team for preventing malignant outcomes from pressure ulcers, pharmacists have been participating in the assessment and treatment of pressure ulcers in the wards and advising medical staff on pharmacological effects, dosage, and adverse effects. They also have to monitor malignancy and managing drugs and dressings.

In this study we developed a simpler procedure that does not stress the patient. To evaluate the usefulness of the method, we examined the correlation between clinical severity and the bFGF level in the wound fluids of patients with pressure ulcers.

MATERIALS AND METHODS

Patients

Wound fluid samples were collected from 6 patients with pressure ulcers during medical treatment sessions. These patients were recruited from the long-term care facility of Ebihara Hospital, Kamogawa, Japan between June 2006 and June 2008. Of these, 2 were males, and 4 were females, with a mean age of 76.8±16.0 years. The most common medical diagnoses of the participants were stroke (n=4), dementia (n=1), and diabetes (n=1).

Quantitative clinical assessment of pressure ulcers

The DESIGN tool for classifying pressure ulcer severity and monitoring the progression towards healing was used to quantify the healing process and enhance the validity of the macroscopic examination (Moriguchi *et al.*, 2002). DESIGN was developed by the Scientific Education Committee of the Japanese Society of Pressure Ulcers: the depth of the pressure ulcers was scored from 0–5, exudate was scored from 0–3, size was scored from 0–6, inflammation/ infection was scored from 0–3, granulation tissue was scored from 0–5, necrotic tissue was scored from 0–2, and pocket was scored from 0–4. Wounds were assessed according to the score for each item and the total score. A decrease in the score indicated an improvement. DESIGN is a reliable tool that provides good reliability with a standard (simple correlation coefficient) of $r = 0.9$ and a high intraclass correlation coefficient with a standard of $r = 0.98$ (Sanada *et al.*, 2004). In the present study, the wound assessment was performed by the same trained researcher to increase the reliability of the assessments.

Wound fluid collection

The wound fluid collection from patients was carried out during regular medical treatment. Sterile gauze (Taketora Cross, Taketora Holdings Co. Ltd., Tokyo, Japan) was placed over the wound for 60 seconds. Wound fluids were then absorbed onto a piece of the sterile gauze by placing it on the most exudative area of the wound. After 60 seconds it was removed from the ulcer base. The absorbed area of the gauze was cut from the non-absorbed area, and the cut piece of gauze was placed into a tube containing sterile phosphate buffered saline (PBS). The gauze that absorbed the fluids was soaked into PBS overnight at 4°C. The soaked buffer was then transferred into a new polystyrene tube and immediately frozen and stored at –70°C until use. The purpose, procedures, and benefits of the project were explained to the participants or their relatives, and at the same time consent was obtained for the study.

Measurement of human bFGF by ELISA

The concentration of bFGF was measured by an enzyme-linked immunosorbent assay (ELISA) as described previously (Okamoto *et al.*, 2009). Microtiter plates (Nunc Immunoplate Maxisorp, Thermo Fisher Scientific, Inc., Roskilde, Denmark) were coated with 50μl of 2μg/ml monoclonal mouse anti-human bFGF antibody (Clone 10060, R&D systems Inc., MN, USA) in carbonate buffer (0.02mol/l, pH 9.5) overnight at 4°C. Thereafter, the wells were blocked with 200μl of phosphate buffered saline (PBS) containing 1% BSA and 5% sucrose for 90 min at room temperature. The plates were then washed three times with PBS containing 0.05% Tween 20 (PBS-T). Recombinant (r) human bFGF (R&D systems Inc. MN, USA), which was used to construct a standard curve (0–2000pg/ml), was serially diluted with 0.5% BSA/PBS-T. Fifty μl of each wound fluid sample were incubated in the wells for 2 hours at room temperature. After the incubation, the plates were washed, and biotinylated monoclonal mouse anti-human bFGF (50μl, 100 ng/ml, clone 10043, R&D systems Inc., MN, USA) was added, before futher incubation for 2 hours at room temperature. The plates were washed, and 50μl of horseradish peroxidase (HRP)-conjugated anti-biotin goat polyclonal antibody (Vector Laboratories, Inc., CA, USA) diluted 1/2000 in 0.05% BSA/PBS-T were added. Incubation was carried out for 1 hour at room temperature. Finally, the substrate (SureBlue TMB Microwell Peroxidase Substrate, KPL, MD, USA) was added and allowed to incubate at room temperature for 3 minutes +/–30 seconds. The reaction was stopped with 2 N H_2SO_4 and the absorbance read at 450 nm was measured in a Microplate reader Model 680 (BioRad Laboratories, CA, USA). Data reduction and the calculation of sample bFGF values were carried out with a statistical software package (Microplate Manager, BioRad).

The protein content of each wound fluid was measured by the method of the Bradford assay with bovine serum albumin as the standard (Bradford, 1976). The bFGF concentrations were corrected for the total protein content of the wound fluids and expressed as pg/mg total protein to exclude the effects of the variations in the dilution with PBS and the recovery rate from the gauze.

Statistical analysis

The Spearman test was used to evaluate correlations between two quantitative variables. A value of $p<0.05$ was considered as statistically significant. The statistical analysis was performed with the aid of the statistical software package Stat View-5.0 (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

The quantitative collection of wound fluids from patients is not easy and furthermore, it is stressful for patients.

In the present study, we performed a simpler procedure that does not cause distress to patients; i.e., sterile gauze was placed over the wound for only 1 minute.

We analyzed the correlation between the bFGF level and clinical severity to test the usefulness of our method. A statistical analysis of the bFGF concentration according to total DESIGN score is shown in **Figure 1**. Spearman's correlation rank

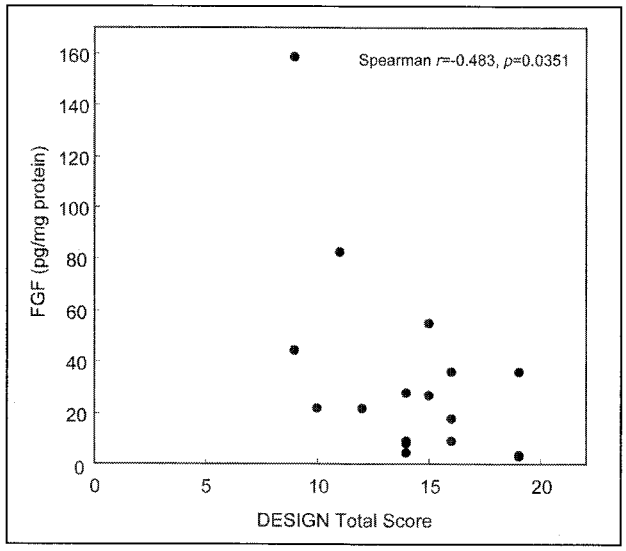

Figure 1. Correlation between bFGF level and the total DESIGN score. Scatter plot demonstrates the significant correlation between the bFGF level in wound fluids and total DESIGN score. bFGF *vs* DESIGN total score: p= 0.0351, Spearman r=-0.483

coefficients (two-tailed) were used to evaluate the relationship between total DESIGN score and bFGF levels in wound fluids. There was a negative statistical significant correlation between them (bFGF *vs* DESIGN total score: p= 0.0351, Spearman r=-0.483). The DESIGN score is a reliable tool for the assessment of clinical severity of pressure ulcers (Sanada *et al.*, 2004). Our results indicate that the bFGF level in wound fluids reflects the clinical severity of pressure ulcers. Thus, the measurement of the bFGF levels of wounds fluids using this simple method may be useful for the objective assessment of severity of pressure ulcers. Furthermore, the relationships between the bFGF level and individual DESIGN score parameters (Exudate, Size, Inflammation/infection, Granulation tissue, Necrotic tissue, and depth) have been evaluated (**Figure 2**). The grades for the depth, the necrotic tissue, and inflammation showed a significant negative correlation with bFGF level in

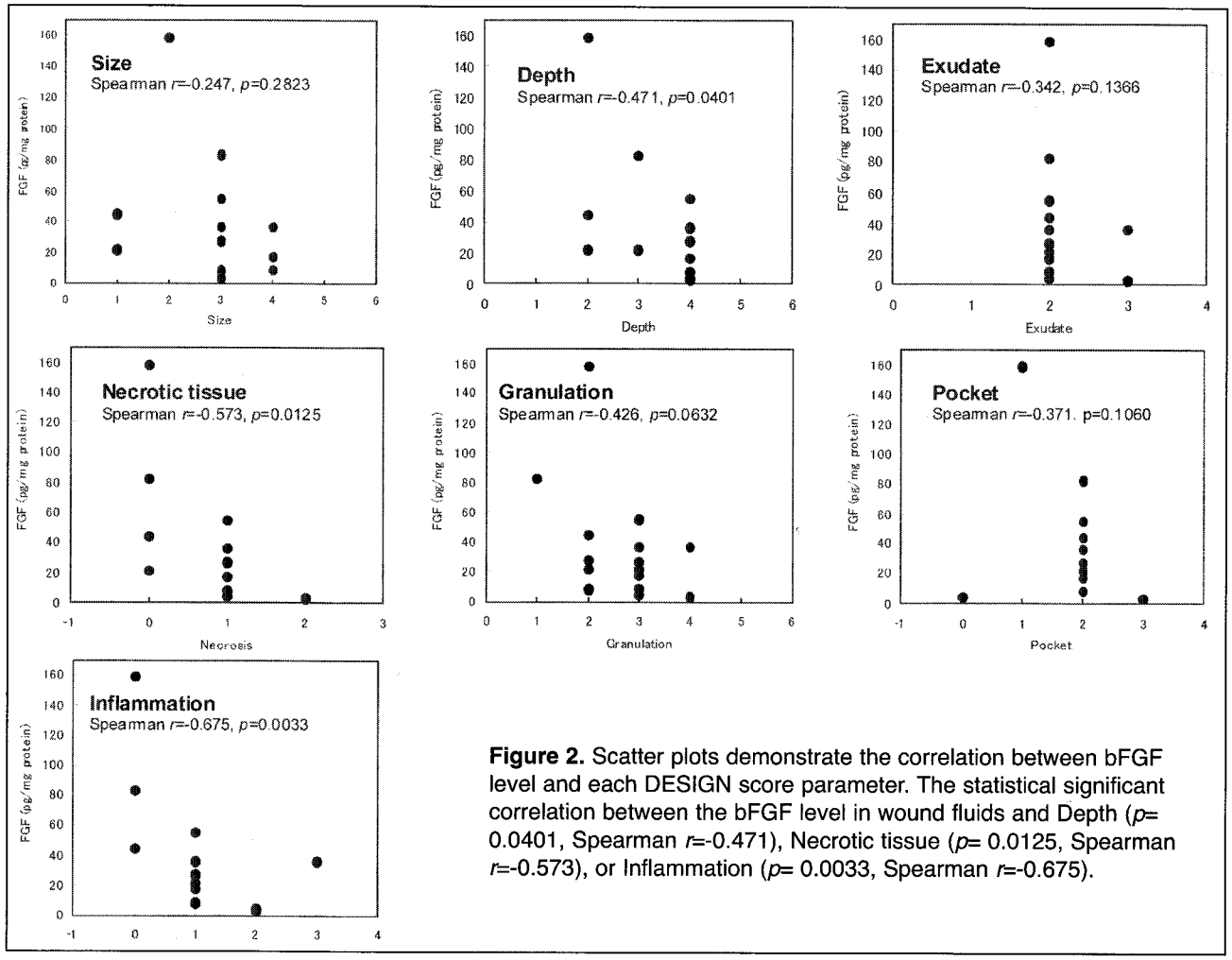

Figure 2. Scatter plots demonstrate the correlation between bFGF level and each DESIGN score parameter. The statistical significant correlation between the bFGF level in wound fluids and Depth (p= 0.0401, Spearman r=-0.471), Necrotic tissue (p= 0.0125, Spearman r=-0.573), or Inflammation (p= 0.0033, Spearman r=-0.675).

wound fluids. The results indicated that the bFGF level at local sites strongly reflects the ulcer depth, necrotic state, and inflammation.

bFGF is a clear candidate for contributing to the wound healing response, and the local application of bFGF stimulates wound repair (Robson *et al.*, 1992, 2000; Werner *et al.*, 2003). However, the bFGF concentration in wounds has not been examined sufficiently. Only Gohel *et al.* have examined the correlation between the bFGF in wounds fluids and clinical severity (Gohel *et al.*, 2008). They demonstrated a negative correlation between the bFGF level in wound fluids and clinical severity (Gohel *et al.*, 2008). Our data were in general agreement with their report.

Although previous studies (Cooper *et al.*, 1994; Trengove *et al.*, 2000; Murphy *et al.*, 2002) did not report any difficulties with wound fluid collection, it is not easy to collect wound fluid from subjects. Most of the failures in sample collection were due to clinical factors; e.g., ulcers did not produce a sufficient amount of fluid to analyze. The other methods of wound fluid collection, such as the use of hydrophilic dextranomer beads (Cooper *et al.*, 1994; Ladwig *et al.*, 2002) or a clear adhesive dressing (Gohel *et al.*, 2008), may result in a greater proportion of successful fluid collection. We assessed values corrected using the protein concentration of the wound fluids to ensure quantitative measurements. The corrected values were correlated with clinical severity.

In summary, this study demonstrated a simple method for the bFGF determination of wound fluids in patients with pressure ulcers, which is useful for the assessment of clinical severity. Moreover, this method should lighten the burden on patients.

Declaration of Interest

The authors declared no financial or commercial conflict of interest.

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