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ORIGINAL ARTICLE

Clinical and Histological Correlation in Post-Burn Hypertrophic Scar for Pain and Itching Sensation

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Background: Hypertrophic scar following a burn is caused by the excessive deposit of collagen resulting in an exaggerated wound healing response. The burn patient complains of pain and itching over the scar, which can give rise to cosmetic and functional problems. **Objective:** The aim of this study was to investigate the clinical and histological correlation of a hypertrophic burn scar for itching and pain sensations. **Methods:** Thirty- eight patients underwent a scar release and skin graft. the modified Vancouver scar scale and the verbal numerical rating scale were recorded. All biopsies were taken from scar tissue (scar) and normal tissue (normal). Histologically, tissues were observed in the epidermis, the monocytes around the vessels, the collagen fiber, elastic fiber, and the mast cells. **Results:** The mean total score of MVSS was 8.4±2.7 (pliability 2.0±0.9; thickness 1.8±0.9; vascularity 2.0± 0.9; and pigmentation 2.1±0.9). Pain and itching were 2.4±2.0 and 2.9±3.0. Epidermis were 7.9±2.8 layers (scar) and 4.0±0.8 layers (normal). The collagen fibers were thin and dense (scar) and thicker and loose (normal). The elastic fibers were thin and nonexistent (scar) and thin and loose (normal). Mast cells were 11.2±5.8/high power field (scar) and 7.4±4.1 (normal). **Conclusion:** As the scar tissue thickens, the itching becomes more severe. The stiffness of the scar with the pain appeared to be associated with the condition of the tissue. The correlation between clinical and histological post-burn hypertrophic scars will help further studies on the scar. This helped with the development of the base material for therapeutic strategies. **(Ann Dermatol 25(4) 428～433, 2013)**

-Keywords-
Hypertrophic scar, Itching, Pain

INTRODUCTION

The extensive loss of skin due to burns is a major human defense mechanism of the skin, where hypertrophic scars remain even after the burn has healed. Postsurgical hypertrophic scars seem to be a common problem reported by surgeons and patients. A hypertrophic scar caused by the excessive deposition of collagen results in an exaggerated wound healing response with a progressive increase in collagen synthesis[1]. Clinically, a hypertrophic scar is defined as an exuberant scar that remains in the area after injury, and leads to itchiness, pain, and a scar with increased thickness, redness, and stiffness[2,3].

An adequate assessment of the scars is important in clinical evaluation and follow-up. It is also important to compare different wound or scar treatment modalities. The modified Vancouver scar scale (MVSS) is commonly used to evaluate scars, but it remains a subjective scar evaluation. The MVSS consists of pliablity, height, vascularity, and pigmentation[4].

The verbal numerical rating scale (VNRS) is a good self-assessment method for pain and is used as an objective assessment method in patients. The VNRS is scored at 0 points when the client experiences no pain and 10 points when the client experiences severe pain[5]. Studies focused on the clinical analysis of hypertrophic scar tissue have not been carried out in Korea, while the histology of scars

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has not been studied due to medical teams' lack of knowledge of the patient's pain and itchiness caused by the neuropathy due to hypertrophic scar tissue. Histologically, the hypertrophic scar of human skin is known to differ from that of normal skin[6].

The aim of this study is to understand the clinical and histological correlation between post-burn hypertrophic scars and the characteristics of hypertrophic scar tissue in earlier Korean studies. The results of our work will help to develop a base for future therapeutic strategies.

MATERIALS AND METHODS

Ethics statement

The protocol was reviewed and approved by the Institutional Ethics Committee for human studies of Hangang Sacred Heart Hospital, Burn Center, Seoul, Korea (2011-092). Each patient was informed of the purpose of the study and informed consent was attained.

Patients and samples

Thirty-seven patients underwent a hypertrophic scar release and skin graft under general anesthesia. The age, gender, days after burn, and injury site of patients varied.

Compared with the original scale, this modified version is a numerical assessment with four skin characteristics, where zero represents the person's normal skin. These characteristics include height (range, 0 to −4), pliability (range, 0 to −4), vascularity (range, 0 to −3), and pigmentation (range, 0 to −3). The investigator assigned each scar site a numerical value for each of these characteristics, based on a comparison with the individual's normal skin. A standard scar measurement protocol was implemented in this study. The MVSS was used as an initial screening tool for hypertrophic scar formation. The MVSS was commonly used in clinics to report the progress of the hypertrophic scar. Scar properties such as pigmentation, height, vascularity and pliability were assessed and rated (Table 1).

Scar thickness was measured using ultrasound (Z One; Zonare Medical System, Mountain View, CA, USA).

Pain and itching over the scar were recorded by VNRS. The VNRS was classified as follows: mild pain (VNRS score 0 to 4), moderate pain (VNRS score 5 to 6), and severe pain (VNRS score 7 to 10). The MVSS and VNRS were measured before the operation. Microscopically, the thickness of the hypertrophic scar, the thickness and density of the collagen fiber (Masson's trichrome stain), the thickness and density of the elastic fiber (elastic stain), and the mast cell count (toluidine blue) were observed. All biopsies were obtained during surgical procedures, with the patient's informed consent between 2011 and 2012. All scar tissue samples were obtained from the burned patients. Control specimens were obtained from areas without a burn. Hypertrophic scar tissue was collected from lesions after the burn injury. The patients did not show evidence of infection or cancer, nor were any patients treated with immunomodulators in the 3 months prior to the surgery. All tissues were placed in 10% neutral buffered formalin for 18 hours, then processed for paraffin embedding with Paraplast (Sigma-Aldrich, St. Louis, MO, USA). Serial 5 μm-thick tissue sections were processed for routine histology.

Histopathological analysis

All specimens were formalin-fixed and paraffin-embedded to prepare hematoxylin and eosin stained slides with Masson's trichrome staining for collagen fibers, Verhoeff's elastic stain for elastic fibers, and toluidine blue stain for mast cells. The thicknesses of the control skin and the hypertrophic scar were measured with a ruler. In a high power view, the layers and thicknesses of the epidermis and the monocyte around the vessels were counted. The thicknesses of individual collagen fibers and elastic fibers were evaluated as either thick or thin. The densities of the collagen fibers and elastic fibers were evaluated as either sparse or dense. The number of mast cells were counted light microscopy using an PlanApo 40 microscope (Nikon Inc., Tokyo, Japan) in ten high-power fields per sample of normal and hypertrophic skin, respectively.

Stastics

The differences between the groups were assessed using the chi-squared test for qualitative parameters and the

Table 1. Modified Vancouver scar scale

Pliability	Height	Vascularity	Pigmentation
0: normal	0: normal	0: normal	0: normal
1: supple	1: 1~2 mm	1: pink	1: hypo
2: yielding	2: 3~4 mm	2: red	2: mixed
3: firm	3: 5~6 mm	3: purple	3: hyper
4: adherent	4: >6 mm		

Spearman's correlation test for correlations (SAS version 9.2; SAS Institute, Cary, NC, USA); results are expressed as mean±standard deviation or standard error of the mean, respectively. *p*-values less than 0.05 were considered significant.

RESULTS

The patients included twenty men and eighty women. The median age of the patients was 26.0 years old (ranging from 1.3 to 78.0 years old). The median period after a burn was 88.0 months (ranging from 3.0 to 360.0 months) (Table 2).

The number of hypertrophic scars was hand (19), foot (3), neck (2), elbow (3), wrist (1), knee (1), thigh (3), arm (3), abdomen (1), axilla (1) and chest (1) (Table 3).

The mean total score of the Vancouver scar scale (VSS) was 8.428±2.781, which included pliability (0.536±0.999), thickness (1.705±1.952), vasculatiry (2.000±0.903), and pigmentation (2.071±0.940). Pain and itching sensation were 2.357±2.059 and 2.857±3.002, respectively (Table 4). With a greater thickness, the sensation of itching increased significantly (Spearman's correlation coefficient=0.337, $p<0.05$). However, the pain did not correlate with the thickness.

A microscopic comparison between a hypertrophic scar and normal skin is summarized in Table 5. The layers of epidermis were 4.000±0.861 (G1) and 7.964±2.848 (G2) ($p<0.001$), and the thicknesses of the epidermis were 0.185± 0.067 cm (G1) and 0.603±0.289 cm (G2) ($p<0.001$). Statistically significant differences were obser-

Table 2. Characteristics of the study population

Variable	Value
Sex (male/female)	20/17
Age (yr)	26.0 (1.3~78.0)
Duration (mo)	88.0 (3.0~360.0)

Values are presented as number or median (range).

Table 3. Site of hypertrophic scar

Site	Number of subject
Hand	19
Foot	3
Neck	2
Elbow	3
Wrist	1
Knee	1
Thigh	3
Arm	3
Abdomen	1
Axilla	1
Chest	1
Total	38

Table 4. Scoring of MVSS and VNRS

Variable	Value
MVSS	
Vascularity	2.000±0.903
Pigmentation	2.071±0.940
Pliablility	0.536±0.999
Height	1.821±0.945
VSS total	8.428±2.781
Us thickness	1.705±1.952
VNRS	
Pain	2.357±2.059
Itching	2.857±3.002

Values are presented as mean±standard deviation. MVSS: modified Vancouver scar scale, VNRS: verbal numerical rating scale, Us thickness: thickness measured with ultrasound apparatus.

Table 5. Pathology data for samples

Variable	G1 (control group)	G2 (scar group)
Epidermis		
Thickness (cm)	0.185±0.067	0.603±0.289*
Layers (layer)	4.000±0.861	7.964±2.848*
Monocytes around vessels (No/HPF)	1.381±0.589	1.571±1.199
Collagen fiber		
Thickness	Thick	Thin
Density	Loose	Dense
Elastic fiber		
Upper dermis	Thin or loose	Thin or none
Lower dermis	Thick & dense	Thin & loose
Mast cell (No/HPF)	7.450±4.174	11.214±5.814

Values are presented as mean±standard deviation, No/HPF: number/high power field, G1: normal grafted tissue, G2: removed scar tissues. *$p<0.001$ compared with G1.

ved between the hypertrophic scar and normal skin. The monocytes around the vessels were 1.571±1.199/high power field (HPF) (G2) and 1.381±0.589/HPF (G1). The count of mast cells was 11.214±5.814/HPF (G2) and 7.450± 4.174/ HPF (G1) ($p<0.05$). However, no statistically significant differences were observed between the hypertrophic scar and normal skin. The collagen fibers were thinner and denser in the hypertrophic scar than in normal skin ($p<0.05$). The elastic fibers were thinner and sparser in the hypertrophic scar than those in normal skin ($p<0.05$). The count of mast cells did not correlate with pain and itching.

DISCUSSION

After a burn injury, the formation of the hypertrophic scar develops with accumulated excessive collagen following the healing of the burn wound. The burn patient complains of pain and itching sensation over the scar tissue, which gives rise to cosmetic and functional problems. The aim of this study was to investigate the clinical and histological correlation of hypertrophic burn scar between pain and itching sensation.

Evaluation of scar using the modified Vancouver scar scale

Hypertrophic scarring usually occurs within 4 to 8 weeks following a wound infection, a wound closure with excess tension, or other traumatic skin injury[7]. It has a rapid growth phase for up to 6 months that gradually regresses over a few years, eventually leading to flat scars with no further symptoms[8,9].

The VSS is commonly used to evaluate scars[10]. A modified version of the VSS, the MVSS, was used for this study. The MVSS is used to assess pliablity, height, vascularity, and pigmentation. Although it provides a useful standardization of scar assessment, the VSS remains a subjective measure.

Patients were recruited from the department of plastic and reconstructive surgery in Korea from December 2010 to December 2012. All patients had received plastic and reconstructive procedures such as a scar release and skin graft for a burn injury. The hypertrophic scars we found appeared more reddish than the normal skin. Different types of scar pigmentation were observed. The scars were yielding and firm on palpation. The mean scar thickness was 1.89 mm among our subjects. The scars appeared to be raised and obvious on the skin surface when compared to normal skin.

Intervention is therefore recommended to minimize the effect of the changing color and thickness on the hypertrophic scar, thus reducing the cosmetic problem.

Pain and itching

Scar formation occurs as part of the multistage wound healing process when body tissues are damaged due to physical injury or impact. In particular, hypertrophic scar formation may occur as a result of a deep burn[11-13]. Hypertrophic scars might be itchy and painful and cause serious functional and cosmetic disability in many burn survivors; almost all burn patients thus complain about the appearance of their scars and suffer from cacesthesia such as itching or pruritus and pain. A previous study reported that the most common and distressful complications of burn patients were abnormal appearance (75.2%), itching (73.3%), and pain (67.6%)[14]. Itching in the hypertrophic scar continues to be a major obstacle in the rehabilitation of severe burn patients. It usually begins at the time of wound closure and then peaks at approximately 3 to 12 months or much later. The deeper the burn, the longer time is needed for it to heal; reepithelialization may increase the risk of developing significant itch. In addition, there may be a relationship between the itch and the site of injury. The itch might further lead to related psychological disturbances such as anxiety, depression, and sleeplessness[15].

In pain and itching sensation of scar among our subjects, we found mean scores of 2.35 and 2.87, respectively, demonstrating mild pain compared to normal skin.

Correlation between verbal numerical rating scale for pain and itching sensation and modified Vancouver scar scale

Pain is an unpleasant sensory and emotional experience, usually associated with actual or potential tissue damage. The definition of itching proposed by Savin[16], it is unsatisfactory because unpleasant is a subjective adjective and is not a descriptor capable of precise definition. Many stimuli are known to induce pruritus[17]. The basic mechanisms of an itch, and the interactions between pain and the itch, have been debated for some time. Nevertheless, there is an obvious differentiation between the neurons involved in the creation of an itch and pain, at least in the peripheral regions[18]. An itch is clearly distinct from pain with respect to the subjective sensation, the inducing stimuli, and the reflex patterns. In contrast to pain-related withdrawal reflexes, itching evokes the characteristic scratching reflex. However, itching and pain share many similarities and are closely related[19,20]. In general, the itch sensation can be reduced by the painful sensations produced by scratching. The inhibition of itching by painful stimuli has been experimentally demonstrated

using various painful thermal, mechanical, and chemical stimuli[21].

The layer hypothesis (elicitation of pain and itch in the periphery area of the scar) suggests that a strong stimulus induces the dermal unmyelinated afferent C-fibers, resulting in the pain sensation; a weak stimulus induces the epidermal unmyelinated afferent C-fibers, resulting in the itch sensation[22]. This study examined the correlation between itching and the other MVSS assessment measures previously mentioned. With the exception of pigmentation, itching correlated with pliability, height, and vascularity. These tools are limited since they only provide subjective data and any useful assessment needs to generate both objective and subjective data.

Histology

The understanding the role of mast cells in scar formation is increasing with new discoveries regarding cell-to-cell communication. Mast cells are an additional leukocyte subset present in the skin, and are an important source of a variety of pro-inflammatory mediators that can promote inflammation and vascular changes[23]. Mediated by the release of soluble mediators such as histamine, heparin, and cytokines, mast cells have been shown to promote fibroblast proliferation[24]. Increased numbers of mast cells during the active period of hypertrophic and keloid scar formation have been reported[25,26]. Clinically, the release of histamine by these cells likely contributes to the common patient complaint of itchiness. In addition, the vasodilatory effect of histamine may promote erythema and leakage of plasma proteins into the regional tissues. Increased numbers of mast cells have also been transiently observed in the normal physiology of cutaneous wound healing. The mast cell population purportedly peaks on days 2 to 3, then steadily returns to normal levels as healing progresses. This decline in mast cell number is in contrast to the cellular events seen in hypertrophic scars, in which increased numbers of mast cells persist indefinitely[27].

An histologic analysis of grafted normal skin and removed scar tissue was carried out and an evaluation was undertaken of the microscopic anatomy epidermis, the monocyte around the vessels, collagen fiber, elastic fiber, and mast cells. In the histologic section, the thickness and layers of the epidermis were thicker when compared to normal skin. The collagen fiber of the scar tissues showed a thin thickness and dense fibers and the elastic fiber of the scar tissues showed either a thin thickness or loose fibers. Mast cells were found in the dermis and we found a greater number of mast cells in the scar tissues, but no correlated pain and itching.

Conclusions

As the scar tissue thickens, the itching sensation becomes more severe. However, the pain did not correlate with itching. Scar thickness was correlated with the layers of epidermis. The stiffness of a scar along with the pain appeared to be correlated to the condition of collagen fiber, which was thin and dense. In addition, the condition of the elastic fiber, which was thin, sparse or nonexistent, also appears to be associated with the stiffness of the scar with pain.

The aim of this study was to understand the clinical and histological correlation of a postburn hypertrophic scar and to develop a base for future therapeutic strategies. Overall, further patient data would be needed for conclusive findings.

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