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Cutaneous Innervation and Itch in Keloids

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Keloids are a form a pathologic scarring consisting of benign overgrowths of dense fibrous tissue. Sensations of itch and pain are common in keloids and were found in 86% and 46% of patients with keloid, respectively, in a previous study (1). The pathogenesis of keloid formation and the reason for the nociceptive sensations are not well understood. An important reason for this is the exclusive occurrence of keloids in humans, which precludes appropriate animal studies. Although histamine release by mast cells, which are found to be increased when keloids were actively forming (2, 3), has been suggested to contribute to itch (4), the use of antihistamines has largely been ineffective in keloids.

We have previously found that itch occurs more frequently at the periphery of keloids whereas pain tends to occur in the center. We have also shown that sensory testing in symptomatic keloids are abnormal, suggesting the presence of small nerve fiber neuropathy (1). In this study, our aim was to examine whether innervation of keloids in the epidermis and dermis is different from healthy skin. We also wanted to determine if there is a difference in innervation between the central and peripheral regions of keloids and if any difference correlated with pain and itch sensory scores.

MATERIALS AND METHODS

The study was approved by the Institutional Review Boards of Wake Forest Medical Center and National Skin Center of Singapore where the subjects were recruited.

Subjects with keloids of more than 6-month duration who were scheduled to have their keloids excised were recruited. Assessment was performed just before surgery and the areas of the keloids in which subjects felt itch and/or pain were marked out. They were asked about their severity of pain and itch when the sensations occurred over the previous one week and to grade them on a 10 cm visual analogue scale (VAS). The VAS is anchored with the verbal descriptors of ‘no sensation’ on the left and ‘the most intense sensation imaginable’ on the right.

Excised keloidal specimens from patients were immediately fixed in 10% neutral buffered formalin for 4–6 h, processed and embedded in wax. Three mm punch biopsies were taken from the central and peripheral region of each keloid specimen and from healthy skin.

Staining of intra-epidermal and dermal nerve fibers was undertaken using the pan-neuronal marker Protein gene peptide (PGP) 9.5. Briefly, ten 10 µm-thick vertical sections were created starting from the center of each biopsy specimen and these were processed for immunofluorescent indirect staining. The sections were labelled with a rabbit polyclonal antibody directed against PGP9.5 (1:2000; Ultraclone, Wellow, UK) and were incubated overnight at +4°C. The sections were then washed and incubated with donkey anti-rabbit biotin-conjugated secondary antibody (1:400; Amersham Bioscience, Buckinghamshire, UK) for 1 h at room temperature. The sections were washed and incubated with streptavidin AlexaFluor594 conjugate (1:400; Molecular Probes, Eugene, USA) for 45 min at room temperature. Subsequently, the sections were washed, mounted with an anti-fade mountant (Vector Laboratories, Burlingame, USA), sealed and examined. For controls, the same protocol was followed except that the primary antibody was omitted.

The sections were photographed entirely as a series of digitized images (TIF-format) using a Leica TS-SP1 confocal microscope (Leica Camera, Solms, Germany) and were subsequently analyzed.

Using a bespoke software, the images were analyzed in columns of 50 pixels width spanning from the stratum corneum into the dermis. The delineation of the epidermal–dermal junction was performed by the analyst. The software divided the columns into boxes of equal height and measurements within each box were taken: area fraction of fluorescent material and statistics on the distribution of fluorescent intensity (sum, mean, standard deviation, skewness and curtosis).

Statistical analysis was carried out using SAS v9.2. The percentage of PGP9.5 stain coverage was calculated by dividing the number of pixels that registered a stain by the total number of pixels present in an area. A mixed model analysis of variance was used to compare the mean values between controls and keloids (overall and those with itch). Spearman’s rank correlation was used to examine the correlation between the VAS itch and pain scores. P-value <0.05 was considered significant.

RESULTS

Thirteen subjects (7 men and 6 women) of Fitzpatrick type IV–VI skin with keloids were recruited (mean age 25 years, range 14–43) and the controls consisted of 9 age-, sex- and site-matched healthy individuals. Nine of 13 had itch and 4 had pain (mean VAS 5.7 and 5.6, respectively). Clear positive PGP9.5 nerve staining was detected in all samples (Fig. 1). Fibres in the epidermis were clearly seen as well as nerve bundles in the papillary dermis. The nerve terminals terminated at different levels in the epidermis, with the highest frequency at the stratum corneum/granulosum interface.

No significant difference in cutaneous nerve density was found between keloids and healthy skin ($p=0.161$) (Fig. 2A) but a trend towards a lower epidermal nerve density was observed in itchy keloids ($p=0.069$) (Fig. 2B). The number of non-itchy keloids was too small to enable a meaningful statistical comparison to be made.

There was no evidence of a difference in nerve density in the papillary dermis and there was no difference in the distribution of nerves between keloids and controls.

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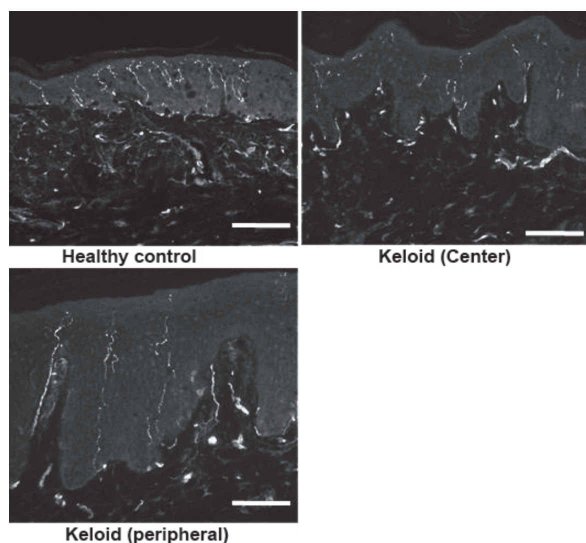

Fig. 1. PGP9.5 staining of nerve fibers in the epidermis and upper dermis. Scale bar = 50µm.

There were no differences in nerve fiber density and distribution in the epidermis between the central and peripheral regions of the keloids. In the dermis, a lower nerve fiber density was noted in the central region of keloids but this was not statistically significant ($p = 0.508$) (Fig. 2C).

There was no significant correlation found between the itch and pain VAS scores and the amount of PGP 9.5 staining in the epidermis and papillary dermis of the central and peripheral regions of the keloids.

DISCUSSION

Previous studies have found that epidermal innervation density is reduced in various pruritic conditions, such as lichen amyloidosis (5), prurigo nodularis (6), and discoid eczema (7). Similarly, in this study, a trend towards a lower epidermal nerve density was found in keloids that are itchy. A hypothesis for these observations may be that chronic stimulation of itch-transmitting nerve fibers results in a self-regulated hypoplasia to modulate the intensity and persistence of sensory input. A reduction in nerve fiber density in the epidermis, where itch-conducting nerve fibers terminate, may therefore be a consequence of chronic pruritus and this phenomenon is generally not observed in the dermis. This observation of likely reduced epidermal nerve fibers also supports our previous study which demonstrated abnormal sensory testing in keloids (1), indicating the presence of small-fiber neuropathy in the disease.

In this study, no correlation between the innervation density of the keloidal skin and the intensity of itch and pain was found. This suggests that the difference in nociceptive sensations experienced in keloids is not due to the difference in the degree of neuronal innervation.

Hypertrophic scars, like keloids, are form of pathological scar and the density of nerve fibers in them were found to be increased in previous studies (8, 9). In a recent study, a greater number of nerve fibers were similarly found in keloids, but these fibers were located deeper in the skin (10). In addition, it was found that those fibers were longer and thinner (10), possibly be due to compression from the thickened and dense collagen fibers and excessive deposition of extracellular matrix in keloids. This compressive effect in the keloidal dermis may yet be another explanation for the trend toward reduced numbers of epidermal nerve fibers observed in this study and may be a contributory mechanism to pruritogenesis in keloids.

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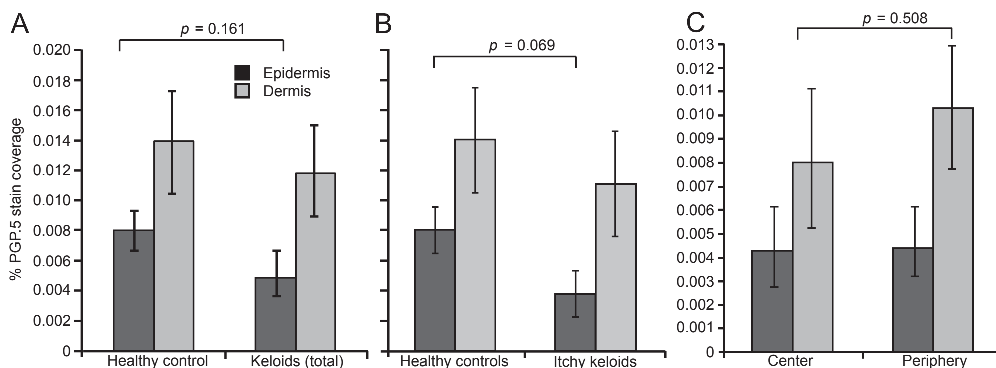

Fig. 2. Percentage PGP 9.5 stain coverage in healthy controls versus keloids, showing no significant differences in nerve fiber density (A) but a trend towards a lower epidermal nerve density when itchy keloids were compared to controls ($p = 0.069$) (B). Mean percentage PGP 9.5 stain coverage in the center versus the periphery of keloids ($p = 0.508$) (C). Values are plotted as mean ± SE.

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