

ORIGINAL ARTICLE

Complete excision of proliferating core in auricular keloids significantly reduces local recurrence: A prospective study

Yosep CHONG,¹ Chan Woo KIM,² Yong Sung KIM,³ Choong Hyun CHANG,³
Tae Hwan PARK² 

¹Department of Hospital Pathology, College of Medicine, The Catholic University of Korea, Seoul, ²Department of Plastic and Reconstructive Surgery, CHA Bundang Medical Center, CHA University, Seongnam, ³Department of Plastic and Reconstructive Surgery, School of Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University, Seoul, Korea

ABSTRACT

Keloids are mysterious soft-tissue tumors that are characterized by excessive reparative processes composed of collagen-forming fibroblasts and inflammatory cells. Generally, complete tumor excision regardless of sufficient margin is considered as a first-line treatment because they are considered reactive rather than a neoplastic condition. Recently, a specific part of the keloids is being highlighted as an important microstructure for local recurrence, but there has been very little evidence. We conducted a prospective study to evaluate the relationship of recurrence and several clinicopathological parameters with specific focus on surgical resection margin. A total 87 cases of auricular keloids from 71 patients were included. The resection margins were carefully evaluated by an exhaustive grossing method and thorough microstructural assessment. During up to 48.8 months of the follow-up period, local recurrence has been monitored and documented. The clinicopathological data including symptoms, bilaterality, size, location, prior treatment and operation history, gross type and etiology were collected and analyzed. Positive margin status was significantly related to tumor recurrence ($P < 0.0001$). Complete excision warrants a lower recurrence of auricular keloids in an Asian population. The most reasonable explanation for this seems to be remnant “proliferating core”, which may serve a key role in local recurrence.

Key words: keloid, local neoplasm, recurrence, prospective study, surgical margin.

INTRODUCTION

Keloid is a benign tumorous condition of the dermis that results from an aberrant reparative process to a local injury, and that is characterized by excessive collagen fiber formation.^{1,2} Pathologically, keloid is considered to be a reactive condition, rather than a neoplastic lesion, for several reasons: First, keloids do not present as confined masses with discreet borders. Second, they are composed of various types of normal cells of heterogenous origins, including fibroblasts and inflammatory cells, rather than being monotonous proliferations of cells of a single origin. Finally, they usually do not present any cytological or structural atypia, unlike benign or malignant neoplasms.

However, the possibility that keloids have neoplastic features has been raised because keloids are frequently related to genetic changes, and local recurrence is commonly observed after resection (in 45–100% of cases),³ resembling characteristics of other neoplastic conditions. It is generally accepted that keloids are triggered by some environmental factors in people with genetic susceptibility. Given the neoplastic feature of keloids such as common local recurrence after resection,

however, we have hypothesized that a group of aberrant cells or specific microstructure may play a key role in the local recurrence of keloids.⁴ If keloids contain any structural elements that have neoplastic features (rather than reactive features), then the frequent recurrence of keloids may be attributable to the blunt and incomplete understanding of this mysterious disease, which often leads to inattentive management of surgical resection margins.^{3,5–7} Indeed, only one study has been performed on the relationship between the resection margin status and local recurrence.⁸

In our previous studies, we simply focused on the findings that local recurrence is closely related to the history of prior surgery in patients with auricular keloids.⁹ Our more recent study on the microarchitecture of auricular keloids suggested that there is a core element to the structure of keloids that is responsible for tumor regrowth and recurrence.⁴ Based on this hypothesis, we designed a prospective study to evaluate the relationship between surgical resection margin status and local recurrence in auricular keloids. We adopted carefully designed grossing method and exhaustive microscopic evaluation for resection margin status to get stronger evidence (Fig. 1).

Correspondence: Tae Hwan Park, M.D., Ph.D., Department of Plastic and Reconstructive Surgery, CHA Bundang Medical Center, CHA University, 59 Yatap-ro, Bundang-gu, Seongnam, Gyeonggi 13496, Korea. Emails: pspark0124@cha.ac.kr or hard-piano@hanmail.net
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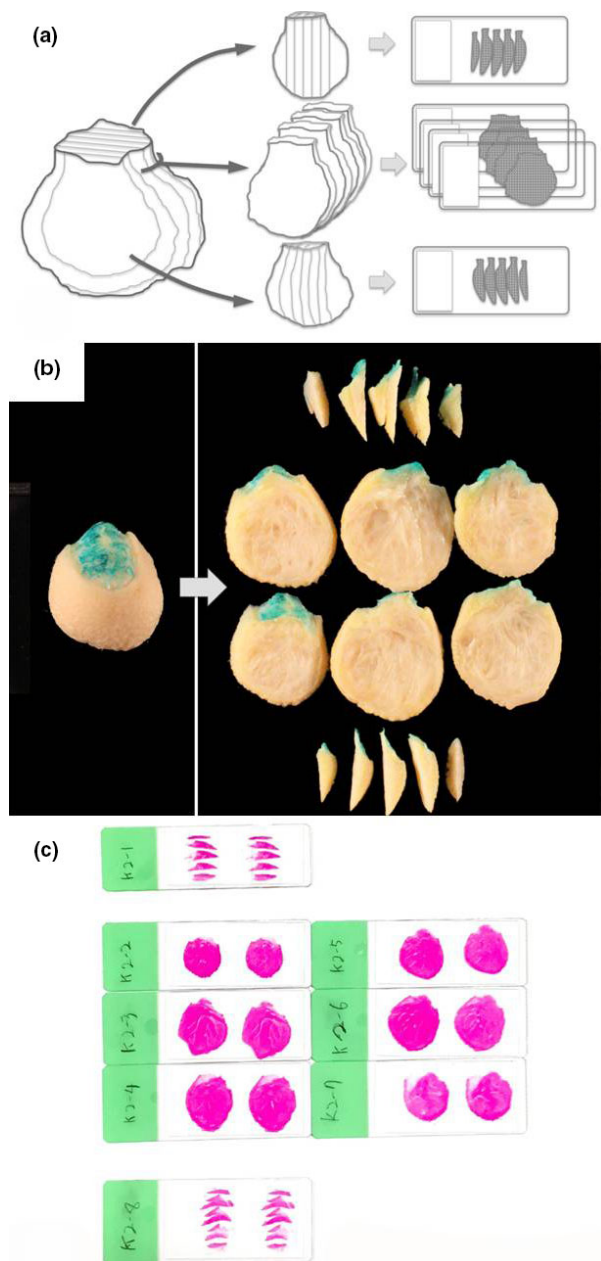


Figure 1. Grossing method and preparation of microscopic slides. (a) Schematic illustration of the gross examination method that was used for evaluating the surgical resected margin status. (b) An example of a sectioned sample. (c) An example of accordingly prepared microscopic slides (hematoxylin-eosin).

METHODS

Patients and samples

This study was reviewed and approved by the institutional review board of our institution. The patients with auricular keloids that were surgically resected from August 2012 to

February 2015 were enrolled in this study. Auricular keloids of types IV (buried type) and V (mixed type) in the Chang–Park classification were excluded because it is difficult to assess the marginal status of the surgical resection accurately in these cases.¹⁰ Likewise, keloids smaller than 0.8 cm were excluded because it is difficult to assess the surgical resection margin status. After applying the exclusion criteria, a total of 87 cases of auricular keloids from 71 patients were included in this study. Clinicopathological data were collected, including symptoms (pain and itching), duration, bilaterality, size (largest diameter), growth rate (size/duration), location (right vs left), treatment (steroid injection) and operation history, gross type (type I, II or III in the Chang–Park classification),¹⁰ etiology (earring or piecing, trauma, or graft for nose augmentation), operation date and follow-up data. Reported recurrence was defined as a further raised scar following complete excision that required second-line treatment, such as re-excision or steroid injection.

Pathological assessment of resected margins of the auricular keloids

The resected specimens were fixed in 10% buffered formalin and investigated using a specially designed grossing method to thoroughly examine the involvement of the keloid collagen fiber on the surgical resection margins, as depicted in Figure 1. The sectioned specimens were embedded in paraffin blocks after a routine preparation process. The paraffin blocks were sectioned at 7- μ m thickness and stained by hematoxylin–eosin (HE) for usual microscopic examination. Van Gieson stain for elastic fibers and Masson-trichrome stain for collagen fibers were performed using the representative sections for each case. The margin status of each case was documented as either positive or negative after reviewing HE stains and special stains. A positive margin was defined as keloid collagen involvement (in most cases, “proliferating core”) on the surgical resection margins for 10% or more of the whole margin surface. A negative margin was defined as keloid collagen involvement (proliferating core) of less than 10%. The keloid collagen component was evaluated as described in our previous study (Fig. 2).⁴

Statistical analysis

The χ^2 -test and Fisher’s exact test were used for the statistical analysis. A *P*-value of less than 0.05 was regarded as indicating a statistically significant finding. Kaplan–Meier survival analysis was performed to assess recurrence-free survival according to the resection margin status.

RESULTS

Clinicopathological features of the enrolled samples

Clinicopathological features of the enrolled samples are summarized in Table 1. The average patient age was 25.6 ± 5.9 years (range, 13–41). Six cases occurred in males and 81 occurred in females (M : F = 1:13.5). The average tumor size was 2.0 ± 0.8 cm (range, 0.8–4.0) and approximately half of the keloids were located in the right ear (47.1%).

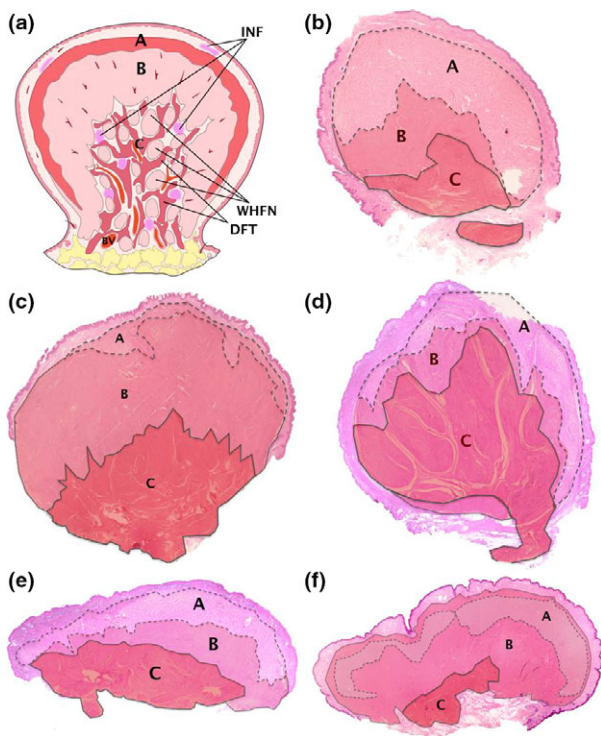


Figure 2. Examples of collagen microarchitecture of the cases with positive and negative resection margins. (a) Schematic illustration of the microarchitecture of auricular keloids (A, keloidal collagen; B, organizing collagen; C, proliferating core collagen; BV, blood vessel; DFT, dense fibrous collagen tissue; INF, inflammatory cell infiltration; WHFN, whorling hypercellular fibrous micronodule). (b) An example case with negative resection margin. (c) An example case with positive resection margin which showed recurrence. (d) A case with deep-rooted proliferating core classified as positive resection margin which later showed recurrence. (e) A broad-based keloid with positive resection margin which showed recurrence. (f) A broad-based keloid with focal involvement of organizing collagen in the resection margin which was classified as negative resection margin and showed no recurrence.

Twelve patients had bilateral lesions (13.8%). According to the Chang–Park classification,¹⁰ the gross types of the cases were I, II and III for 14, 44 and 29 cases (16.1%, 50.6% and 33.3%), respectively. At the time of surgery, the patients had had the lesions for an average of 33.8 months (range, 3–120). Three cases (3.5%) did not include any specific symptoms, while 30 cases (40.5%) included pain and 58 cases (78.4%) included itching. Nineteen cases were treated with a previous operation (21.8%). Thirty-nine cases were previously treated with steroid injection (45.3%). An average of 2.6 paraffin blocks (range, 1–6) were prepared and used to assess resection margins. Only 16 cases had free resection margins (18.4%). The cases were followed up for an average of 19.8 months (range, 1.2–48.8). Tumor recurrence was found in 18 cases (20.7%). No tumor recurrence was found in cases with free margins, whereas all 18 recurred cases had positive resection margins.

Clinicopathological parameters related to recurrence

Clinicopathological parameters were compared between cases that did and did not recur. Average age did not differ significantly between the two groups (24.6 vs 25.9 years, $P = 0.193$). The sex distribution did not differ significantly between the groups either ($P = 0.599$). The average tumor size was slightly smaller (1.7 ± 0.5 vs 2.0 ± 0.8 cm) in the recurrence group, but the difference was not statistically significant ($P = 0.253$). The groups did not differ significantly in terms of keloid location (right or left lesion) ($P = 0.798$). Contrary to our expectations, bilaterality was not associated with recurrence ($P = 1.000$), meaning that the patients with bilateral lesions did not show significantly more recurrences than the patients with one-sided lesions. Furthermore, the gross types of the lesions were not significantly associated with recurrence ($P = 0.564$). The average age of keloids was slightly shorter in the recurrence group than in the non-recurrence group (22.7 ± 16.5 vs 36.8 ± 31.4 months), but the difference was not statistically significant ($P = 0.072$). The growth rate, which was derived by dividing size by age of keloid, was slightly higher in the recurrence group (0.14 ± 0.11 vs 0.12 ± 0.15 cm/month), but the difference was not statistically significant either ($P = 0.558$). Regarding the etiologies of the lesions, cases that occurred after trauma or nose augmentation were more associated with recurrence than were cases that occurred after earring or piercing, although the difference was not statistically significant ($P = 0.080$). The presence of any symptoms did not show any significant relationship with recurrence ($P = 0.499$), and neither did pain or itching alone ($P = 0.962$ and 0.291 , respectively). Prior operation history and prior steroid injection treatment history before the surgery were not related to recurrence ($P = 1.000$ and 0.184 , respectively). Finally, margin involvement status was strongly associated with recurrence ($P = 0.035$). Recurrence was not observed in any case with negative resection margins, and all recurred cases had positive resection margins (Fig. 2).

Kaplan–Meier survival analysis

A Kaplan–Meier survival analysis also clearly showed the relationship between surgical margin status and recurrence, as depicted in Figure 3. During the entire follow-up period, no recurrence was seen among the cases with negative resection margins. On the other hand, the cases with positive resection margins showed significantly shorter recurrence-free survival.

DISCUSSION

Relationship between recurrence and surgical resection margin

The single most important finding of this study is the significant relationship between the surgical resection margin and recurrence ($P = 0.035$). More precisely, all the recurred cases had positive resection margins, and none of the cases with negative resection margins recurred. We can draw two important messages based on these results, which are similar to the findings of Tan *et al.*'s⁶ study in the UK, in which keloids that occurred in various sites were examined.

Table 1. Clinicopathological parameters and their relations to recurrence

Clinicopathological parameters	Value (%)	Cases with recurrence	Cases without recurrence	<i>P</i>
Age (years)				
Average	25.6 ± 5.9	24.6 ± 6.6	25.9 ± 5.6	0.193
Range	13–42	18–42	13–40	
Sex				
Men	6 (6.9%)	2 (11.1%)	4 (5.8%)	0.599
Women	81 (93.1%)	16 (88.9%)	65 (94.2%)	
Tumor size (cm)				
Average	2.0 ± 0.8	1.7 ± 0.5	2.0 ± 0.8	0.253
Range	0.8–4.0	1.1–3.0	0.8–4.0	
Tumor location				
Right	41 (47.1%)	8 (44.4%)	33 (47.8%)	0.798
Left	46 (52.9%)	10 (55.6%)	36 (52.2%)	
Bilaterality				
Present	12 (13.8%)	2 (11.1%)	10 (15.9%)	1.000
Absent	75 (86.2%)	16 (88.9%)	59 (84.1%)	
Gross type				
I	14 (16.1%)	1 (5.6%)	13 (18.8%)	0.564
II	44 (50.6%)	11 (61.1%)	44 (47.8%)	
III	29 (33.3%)	6 (33.3%)	29 (33.3%)	
Age of keloid (months)				
Average	33.8 ± 29.5	22.7 ± 16.5	36.8 ± 31.4	0.072
Range	3–120	4–60	3–120	
Growth rate (cm/month)				
Average	0.13	0.14 ± 0.11	0.12 ± 0.15	0.558
Range	0.02–0.74	0.02–0.35	0.02–0.74	
Etiology				
Unknown	9	2	7	
Earring or piercing	69 (82.6%)	12 (75.0%)	57 (91.1%)	0.080
Trauma or nose augmentation	9 (11.5%)	4 (25.0%)	5 (8.1%)	
Symptoms				
Unknown	13	3	10	
Absent	3 (4.1%)	1 (6.7%)	2 (3.4%)	0.499
Present	71 (95.9%)	14 (93.3%)	57 (96.6%)	
Pain				
Absent	44 (59.5%)	9 (60.0%)	35 (59.3%)	0.962
Present	30 (40.5%)	6 (40.0%)	24 (40.7%)	
Itching				
Absent	16 (21.6%)	5 (33.3%)	11 (18.6%)	0.291
Present	58 (78.4%)	10 (66.7%)	48 (81.4%)	
Operation history				
Yes	19 (21.8%)	4 (22.2%)	15 (21.7%)	1.000
No	68 (78.2%)	14 (77.8%)	54 (78.3%)	
Prior steroid treatment				
Yes	39 (45.3%)	11 (61.1%)	28 (41.2%)	0.184
No	47 (54.7%)	7 (38.9%)	40 (58.8%)	
Examined paraffin blocks				
Average	2.6 ± 1.2	2.4 ± 1.1	2.7 ± 1.2	
Range	1–6	1–5	1–6	
Margin involvement*				
Present	71 (81.6%)	18 (100.0%)	53 (76.8%)	0.035*
Absent	16 (18.4%)	0 (0.0%)	16 (23.2%)	
Follow-up period (months)				
Average	19.8 ± 9.0	14.4 ± 10.0	20.9 ± 8.3	
Range	1.2–48.8	1.5–28.5	1.2–48.8	
Recurrence				
Present	18 (20.7%)			
Absent	69 (79.3%)			

Asterisk and bold denote *P*-value of less than 0.05 was regarded as indicating a statistically significant finding.

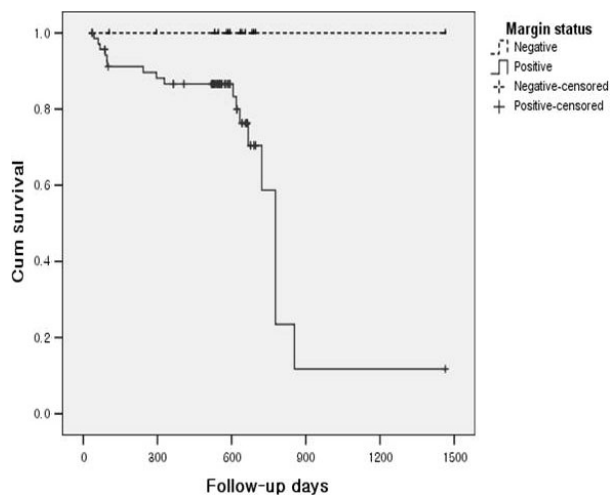


Figure 3. Kaplan–Meier survival analysis for recurrence according to resection margin status.

First, thorough assessment of the marginal status of keloids is important when surgical resection is being used for the first-line treatment. For most surgeons who deal with keloids, there is an inevitable pressure to minimize defects by reducing resection, to preserve aesthetic function as much as possible. Furthermore, the whole idea that keloids are just aberrant reactive lesions can prompt surgeons to leave some tissue, instead of removing all the tumor tissue with proper resection margins (as would be done for other neoplasms). However, our study clearly showed that none of the auricular keloid cases with sufficiently resected margins recurred.

Second, although it is generally accepted that keloids are non-neoplastic lesions, we hypothesize that there is an important microstructure that serves as a proliferating core for tumor growth and local recurrence (Fig. 2). In our previous study, we categorized keloid architecture into three distinct collagen structures by morphometrically comparing the fibroblast cellularity, blood vessel density and the inflammatory cell infiltration degree between each part.⁴ The proliferating core is located in the deepest portion of the auricular keloids and consists of whorling hypercellular fibrous micronodules/fascicles and intervening loose connective tissue with the abundant vasculature. The fibroblast cellularity, blood vessel density, inflammatory cell infiltration, and mast cell counts were significantly increased in the proliferating core, as compared with the other part of the collagen architecture of the auricular keloids. In 2001, Luo *et al.*¹¹ reported a lower rate of apoptotic cell death in the fibroblasts of the deep portion of the keloids. Similarly, Supp *et al.*¹² found that deep and superficial keloid fibroblasts had significantly different expressions of several genes that are implicated in keloid scarring. In 2011, Syed *et al.*¹³ also showed that there were significantly different levels of mRNA expression of collagen I and III between the different lesional sites of the tumor (perilesional, intralesional and extralesional). They designated the growing margin as the main culprit for keloid scarring. However, their and other studies lacked

thorough evaluations of histological architecture, and did not consider the fact that the collagen microstructure is much more complex for keloids occurring in body parts other than the ears. This finding strongly suggests the need for routine pathological examination of the surgical resection margins, unlike a previous study.⁵ A study by Tan *et al.*⁶ also commented that routine pathological examination was needed, and additionally reported that the relationship between local recurrence and the presence of infiltrative keloid border was more obvious in Afro-Caribbean and Asian subgroups.

Relationship between recurrence and other clinicopathological parameters

All other clinicopathological factors were not significantly related to recurrence in the present study. To minimize the confounder bias that can be derived from other factors than the resection margin status, we excluded keloids smaller than 0.8 cm or of gross type IV and V (buried and mixed type) at study design. Given those inclusion criteria, no significant relationships between recurrence and size, the age of keloids and growth rate are reasonable findings in the present study. In 2011, our group performed a large-scale descriptive study using the clinicopathological data of 1436 auricular keloids.⁹ Among all the clinicopathological parameters, slower growth rate, prior treatment history and higher body mass index were statistically related to the recurrence. Synthesizing the findings of present and previous studies, we can presume that the keloids that are easily recurring are generally old, slowly growing and have a complex microstructure that may be rooted deeply, which may lead to an incomplete resection in the deep portion. Finally, the remnants of the prior surgery grow again over time. In 2015, our group also conducted a retrospective histopathological study that analyzed the relationship between recurrence and the pathological parameters including resection margin status.⁸ However, due to the retrospective nature of the study, we could not accurately estimate margin involvement in more than half of the cases and the level of assessment was also limited because only representative sections were available for microscopic review (margin status was unknown in 22 out of 38 cases). To overcome this limitation, we adopted a prospective study design and exhaustive gross examination method in the present study.

Regarding the etiologies of the lesions, the recurrence group showed slightly greater proportions of cases that occurred after trauma or earlobe cartilage graft harvesting for nose augmentation, as compared with cases that occurred after earring or piercing punctures. This indirectly reflects the nature of the keloids in terms of their etiologies. The study by Tan *et al.*⁶ also showed no specific relationship between any other clinicopathological parameters and recurrence.

A large number of cases did not show recurrence, even though they had positive resection margins during the follow-up period (76.8%). This finding leaves a pending question that there may be another factor that may affect local recurrence in addition to margin status. The included cases were restricted to keloids larger than 0.8 cm and type I, II and III in a Korean (Asian) population and this may be another thing to be considered when applying the results of this study.

In conclusion, this prospective study has clearly demonstrated that a negative surgical margin status decreased local recurrence. Therefore, for auricular keloids in Asian populations, complete excision warrants a lower rate of recurrence. The most reasonable explanation for these findings seems to be remnant proliferating core, which may serve a key role in local recurrence. Clinicians who plan for surgical excision as a first-line treatment should be aware of the importance of complete removal of the proliferating core and thorough pathological examination.

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CONFLICT OF INTEREST: None declared.

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