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The Association of Keloid Site with its Histopathological Features: an Analytical Observational Study

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ABSTRACT

Background: Keloid is a growth of fibrous tissue in the wound tissue of susceptible individuals. This tissue extends beyond the boundaries of the previous wound. The site of keloids commonly appears on a high-tension area, such as the chest, shoulders, and neck. Histopathologically keloids show thickened the epidermis and the vascularization and infiltration of inflammatory cells in the dermis. **Purpose**: This study aims to determine the relationship between the site of keloids and the histopathological appearance of keloids. **Methods**: An analytical observational study was conducted on keloid patients visiting the Dermatovenereology outpatient clinic of Dr.Moewardi Hospital. The sample collection used consecutive sampling techniques **Result**: The majority of keloid patients are >30 years old (46.4%). Most patients with keloids were female (53.6%). Keloids were mostly found on the chest (25.0%). Tounge-like appearance of the epidermis at the edges of the lesions was mostly on the shoulders and chest (33.3% each, p=0.048); flattened appearance was found on the middle epidermis of the lesions, which were mostly on the ears, shoulders, and upper extremities (22.7% each, p=0.011). Increased vascularity was found in the dermis at the edges of the lesions, especially in the ear and chest areas (31.3% each, p=0.046). Moderate-severe inflammatory infiltrates in the dermis at the edges of the lesions were commonly found on the chest (p=0.04). **Conclusion**: There is a significant relationship between the site of the lesion and the histopathological appearance of the keloid in epidermal as well as dermal layers.

Keywords: keloid, histopathological, site, epidermis, dermis.

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BACKGROUND

Keloids are benign growths of fibrous tissue characterized by excessive accumulation of collagen and increased fibroblasts.[1] Keloids occur as a result of abnormal wound healing, but their exact mechanism remains unknown.[2,3,4] The prevalence of keloids can be influenced by geography and ethnicity. Averagely the incidence of keloids is estimated to be only 5-10% in Africa and 0.1% in Asia.[5] Keloids can occur at any age, but they tend to be more common between the ages of 11 and 30.[4] A study by Dharmawan reported that the age range of keloid patients was between 21-30 years with the majority being female.[6] The clinical characteristics of keloids are the growth of lesion into the surrounding normal skin beyond the wound's edge, accompanied by itching and pain. Keloids also show a tendency to reappear after surgical excision without adjuvant therapy.[7] Several factors can trigger keloids, such as wounds or trauma on anatomical sites that are prone to keloids (topological factors), genetic factors, and individuals who have dark pigmented skin types.[9,10]

In general, the site of keloids can occur in areas with high mobility and tension, such as the chest, shoulders, and neck.[8,11] High-tension area affect the growth of keloids; for example, keloid lesions in the chest will be formed symmetrically like a butterfly due to stretching from the movement of the upper arms, while on the ears, the growth of keloid lesions is caused by nocturnal friction on a pillow.[5] The site of the keloid shows that mechanical stimulation not only promotes keloid growth but is also responsible for keloid formation.[14]

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The pathogenesis of keloids is thought to be caused by a prolongation of the inflammatory phase, which results in increased release of cytokines and growth factors, stimulating fibroblast proliferation, and excessive deposition of extracellular matrix. Cells that play a role in this process are fibroblasts, myofibroblasts, macrophages, lymphocytes, and mast cells.[12] The formation of neovascularization, along with an increase in inflammatory cells, is the effect of mechanical stimulation on body areas with high tension.[13] Ogawa hypothesized that keloids result from an exaggerated inflammatory response by a strong mechanical stimulus in high-tension areas.[14]

Histopathological features of keloids include thickened epidermis as well as vascularization and infiltration of inflammatory cells in dermis. A study by Samaka found an increase in epidermal thickness in keloids. Rete ridge or tongue-like formation and flattening of the epidermis, accompanied by an increase in thickness are also found on histopathological examination of keloids. Histopathological studies of the keloid dermis show the presence of excessive fibroblasts and inflammatory cells.[9] Hence, we conducted a study to determine the relation between the site of keloids and the histopathological appearance of keloids to provide knowledge about the role of mechanical stimulation at the keloid site on keloid growth.

METHODS

We conducted an analytical observational study on keloid patients visiting the dermatovenereology outpatient clinic of Dr. Moewardi Hospital, Surakarta, Indonesia. The consecutive sampling technique was applied for the sample collection. To determine the sampling formula, researchers refer to the opinion of Ridwan and Akdon, namely using the Lemeshow formula which is used to determine the minimum sample size required in quantitative research, if the population is unknown or unlimited.[24]

$(Z\alpha^2PQ)/d^2 = (1.96^2 x\ 0.5x0.5\ )/0.2^2 = 24$

Z = 1.96 (=0.05).

P = proportion of patients at risk of keloids, because the magnitude is not yet known, P = 0.5,

Q = 1-P=1-0.5=0.5;

d = relative level of accuracy, d value is set = 0.2.

Based on the sample size calculation above, the minimum sample size was 24 patients. To prevent drop outs in the study which resulted in a reduction in subjects, the researchers added 15% of the subjects so that the total number of subjects was 28. We included new keloid patients who had never received treatment and whose keloids had been existing for 6 months, and we excluded those with pregnancy, hormonal contraception, and secondary infections. The keloid samples were obtained from the edges and the middle of the lesions using a 5 mm punch biopsy. A histopathological examination was performed with hematoxylin and eosin (H&E) by a pathologist at the Anatomical Pathology Faculty of Medicine, Sebelas Maret University, Surakarta. The data obtained were statistically analyzed with SPSS for Windows version 22.0. The significance was determined at $p<0.05$. Ethical clearance was obtained from the Ethical Committee of Dr. Moewardi General Hospital, with a reference number of 176/II/HREC/2022.

The tissues were embedded in paraffin blocks, then cut and placed on the slide. Deparaffinization of the samples used xylene solution, then hydrated them in a series of graded alcohol solutions until finally used sterile water. Haematoxylin and eosin staining was done for a few minutes, then rinsed with sterile water and covered them with a cover slip.

RESULT

We obtained 28 keloid samples who met the inclusion criteria. The majority of these patients were over thirty years old (46.4%). Keloids were more common in females (53.6%) than in males (46.4%). Keloids were mostly found on the chest (25.0%), followed by ears, shoulders, and upper extremities (Table 1).

The histopathological examination of the study samples revealed that in the epidermis there were tongue-like appearances which were mostly found at the edge of the lesion (42.9%) and flattened epidermis on the center of the lesion (78.6%). In addition, there was an increase in vascularization (57.1%), along with mild and moderate inflammatory infiltrations at the center (85.7%) and the edge of the lesions (57.1%), respectively. (Table 2; Figure 1).

Table 1. The demographic characteristics of the study subjects

Characteristic	Number (28)	Percentage (%)
Age		
< 20 years old	7	25.0
20-30 years old	8	28.6
>30 years old	13	46.4
Sex		
Male	13	46.4
Female	15	53.6
The keloid's site		
Ears	5	17.8
Shoulders	5	17.8
Chest	7	25.0
Back	3	10.7
Upper extremities	5	17.8
Lower extremities	3	10.7

Table 2. Histopathological features of keloids in the study subjects

No.	Histopathology	Edge of lesion (n=28)	Percentage (%)	Center of lesion (n=28)	Percentage (%)
1	Epidermis:				
	Tounge-like	12	42.9	6	21.4
	Flattened	16	57.1	22	78.6
2	Dermis:				
	Vascularization				
	Normal	12	42.9	21	75
	Increased	16	57.1	7	25
	Inflammatory infiltration				
	Mild	10	35.7	24	85.7
	Moderate	16	57.1	4	14.3
	Severe	2	7.1	0	0

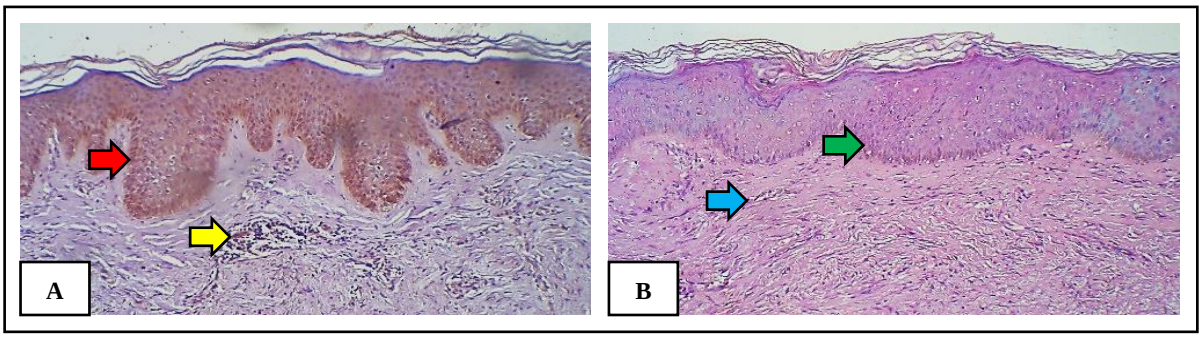

Figure 1. Hematoxyline & Eosin staining of the keloids. (A) Perilesional keloid showing: tongue-like appearance in the epidermis (red arrow) and increased microvasculare as well as inflammatory cell infiltrates (yellow arrow). (B) Intralesional keloid demonstrating flattened epidermis (green arrow) with the dermis showing reduced microvascular and inflammatory cell infiltrate (blue arrow).

Table 3. The relationship between the site and histopathological features of keloids in the epidermis

Site of keloids	Edge of lesion in epidermis			Center of lesion in epidermis		
	Tongue-like	Flattened	P	Tongue-like	Flattened	P
Ear	0 (0.0%)	5 (31.3%)	0.048*	0 (0.0%)	5 (22.7%)	0.011*
Shoulder	4 (33.3%)	1 (6.3%)		0 (0.0%)	5 (22.7%)	
Chest	4 (33.3%)	3 (18.8%)		3 (50.0%)	4 (18.2%)	
Back	0 (0.0%)	3 (18.8%)		3 (50.0%)	0 (0.0%)	
Upper extremities	1 (8.3%)	4 (25.0%)		0 (0.0%)	5 (22.7%)	
Lower extremities	3 (25.0%)	0 (0.0%)		0 (0.0%)	3 (13.6%)	
Total	12 (42,9%)	16 (57,1%)		6 (21,4%)	22 (78,6)	

Note:* Significant

Based on the Chi Square test, a significant association was obtained between the site of keloids and the histopathological features of keloids in the epidermis, both in periphery (p=0.048) and the center of the lesions (p=0.011). At the edge of the lesion, tongue-like appearance was more commonly seen on the shoulders and chest, while flattened epidermis was common on the ears. Meanwhile in the center of the lesion, a tongue-like appearance was mostly on the chest and back. Flattened epidermis was more common than a tongue-like appearance (Table 3).

At the edge of the keloid lesion, increased vascularization was more common than the normal one (p=0.046), and the most common sites of keloid with increased vascularization in the dermis were the ear (31.3%) and chest (31.3%). Regarding the center of keloid lesions, 75% of these lesions had normal vascularization (p=0.026) and increased vascularization was mostly observed in keloid occurring on the back (42.9%). (Table 4)

Table 4. The relationship of the site of keloids with vascularization in the dermis

Site of keloids	At the edge of the lesion			At the center of the lesion		
	Normal	Increased	P	Normal	Increased	P
Ear	0 (0.0%)	5 (31.3%)	0.046*	4 (19.0%)	1 (14.3%)	0.026*
Shoulder	4 (33.3%)	1 (6.3%)		5 (25.8%)	0 (0.0%)	
Chest	2 (16.7%)	5 (31.3%)		6 (28.6%)	1 (14.3%)	
Back	1 (8.3%)	2 (12.5%)		0 (0.0%)	3 (42.9%)	
Upper extremities	2 (16.7%)	3 (18.8%)		3 (14.3%)	2 (28.6%)	
Lower extremities	3 (25.0%)	0 (0.0%)		3 (14.3%)	0 (0.0%)	
Total	12 (42.9%)	16 (57.1%)		21 (75%)	7 (25.0%)	

Note:* Significant

Table 5. The relation between keloid site and inflammatory infiltrate in the dermis

Site of keloids	At the edge of the lesion			At the center of the lesion		
	Mild	Moderate	Severe	Mild	Moderate	Severe
Ear	1 (10.0%)	4 (25.0%)	0 (0.0%)	2 (8.3%)	3 (75.0%)	0 (0.0%)
Shoulder	1 (10.0%)	4 (25.0%)	0 (0.0%)	5 (20.8%)	0 (0.0%)	0 (0.0%)
Chest	2 (20.0%)	3 (18.8%)	2 (100%)	7 (29.2%)	0 (0.0%)	0 (0.0%)
Back	1 (10.0%)	2 (12.5%)	0 (0.0%)	3 (12.5%)	0 (0.0%)	0 (0.0%)
Upper extremities	4 (40.0%)	1 (6.3%)	0 (0.0%)	5 (20.8%)	0 (0.0%)	0 (0.0%)
Lower extremities	1 (10.0%)	2 (12.5%)	0 (0.0%)	2 (8.3%)	1 (25.0%)	0 (0.0%)
Total	10 (35.7%)	16 (57.1%)	2 (7.1%)	24 (85.7%)	4 (14.3%)	0 (0.0%)
P	0.004*			0.026*		

Note:* Significant

Regarding inflammatory infiltrates in the dermis of the edge of the lesion, mild inflammation mostly occurred in the upper extremities (40.0%), moderate inflammation was dominant in ears and shoulders (25% each), and severe inflammation was only found in the chest of two subjects. Meanwhile, in the center of the lesion, mild inflammation was mostly observed on the chest (29.2%), moderate inflammation was predominant in the ears (75%), and there was no severe inflammation. Thus, the site of keloid is significantly related to inflammatory infiltrates at the edge and center of lesions (p=0.04 and p=0.026, respectively). (Table 5)

DISCUSSION

Keloids are pathological wounds marked by excessive production of fibroblast and collagen in the dermal layer.[5] Keloids may occur in both males and females at any age, but mostly they appear between the ages of 11 and 30 years old.[4] However, other studies reported that the incidence of keloids is higher in females than males.[1,15] A study by Dharmawan in 2019 described that the majority of keloid patients were females aged between 21 and 30 years old.[6] In this study, keloids were mostly found in females aged over 30 years old, which is in line with the previous study by Lu in 2015 reporting that the predominance of keloid subjects was female and mostly in the age range of 10 to 30 years old.[17]

The predilection site of keloid is a high-tension area, such as the chest, back, and shoulders.[16] We also found that keloids were predominantly observed on the chest. This finding is in accordance with the Huang et al. study in 2020, in which keloids were dominantly seen on the chest, the high-tension area.[5] The high tension-induced mechanical stimulus triggers increased production of extracellular matrix, vascularization, and macrophage activity.[16]

Histopathologically, keloids are marked by thickened epidermis in the form of rete ridges or tongue-like or flattened area, while in the dermal layer there is an increase in perivascular inflammatory cell infiltrates, along with enhanced vascularization.[18] Samaka et al. study described that in the dermal layer there are more inflammatory infiltrates at the edge of the keloid lesion than in the center one.[2] Our histopathological findings are similar to those of the previous studies, in which thickened epidermis with a tongue-like appearance mostly appear at the edge of the lesion, while flattened epidermis is predominantly seen in the center of the lesion. This indicates that keratinocyte epidermis plays a role in the pathogenesis of keloid. We also found more inflammatory infiltrates and vascularization at the edge of the lesion than in the center of the keloid lesion. A previous study also revealed that the edge of a keloid lesion has progressive growth characterized by increased vascularity as well as active inflammatory infiltrates.[18]

Mechanical stimulation of the keloid tissue on the high-tension skin affects the proliferation of endothelial cells and cytokine secretion, resulting in a diameter alteration of the vessel lumen of the keloid lesion.[14] This study suggests a significant relationship between the site of keloid and dermal vascularization, whereas vascularization was more common at the edge of the lesion, as this site has more robust neovascularization compared to the center of the keloid lesion, which tends to have fewer vessels, leading to ischemia.[13,19] Most of our study subjects had keloids on their ears and chests with vascularization features on histopathological examination. Another study by Matsumoto revealed that there is an increase in vascularization surrounded by collagen hyalinization in the papillary dermis of ear keloids.[20]

The growth of the edge of a keloid lesion is more active in high-tension areas.[13] This induces the augmentation of inflammatory cells and growth factors such as macrophages, mast cells, neutrophils, lymphocytes, and vascular endothelial growth factor (VEGF), inducing cell proliferation, angiogenesis, and collagen synthesis.[16] In this study, moderate and severe inflammatory infiltrates were observed more at the edge of the lesion (chest, shoulders, and ears) than in the center of the lesion, which is in accordance with the Jiao et al. study reporting moderate perivascular chronic inflammatory infiltrates in all keloids.[21] Another study described increased inflammation at the edge of the lesion, which then gradually declined, toward the center of the lesion.[22] The keloid predilection site relates to the amount of inflammatory cells produced. Chest, ears, chin, neck, and shoulders have more inflammatory cells compared to other body areas, such as eye lids, cheeks, and abdomen.[23]

This study was limited by a short study period, as it was done in a year, so the estimated prevalence is low. Additionally, since this is a single-center study, the sample size is small. Further study of longer period and a large sample size is required in order to generalize the outcomes of the study.

The site of the keloid lesion relates significantly to the histopathological features of the epidermis and dermis of keloids.

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