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CORRELATION CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EXPRESSION OF INTERLEUKIN-6 (IL-6) IN KELOID

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ABSTRACT
IL-6 has been shown to enhance collagen synthesis and prolong the fibroblast proliferative phase, potentially contributing to uncontrolled keloid growth. However, despite its frequent mention in the literature as a crucial factor in keloid formation, studies specifically exploring the molecular mechanisms of IL-6 in fibroblast regulation and its interaction with other cytokines within keloid tissue remain limited. This study aims to further investigate the role of IL-6 in keloid matrix formation. This study is an analytical study conducted cross-sectionally. The samples used were patients diagnosed with keloids histopathologically based on microscopic criteria. Clinical data were taken from medical records and microscopic and immunohistochemical assessments using paraffin blocks available in the anatomical pathology laboratory of hospitals in Jambi city. There were 36 cases of keloids, aged between 12 and 42 years, mostly in women (66.7%), occurring in the trunk area (77.8%) 30 cases of recurrence (83.3%). We found 24 cases (77.8%) with strong IL-6 immunoexpression. There were 3 cases (8.3%) with no immunoexpression. There was a strong correlation between IL-6 immunoexpression and gender (p=0.03), age(0.04) and recurrence (p=0.02). The role of fibroblast cells is very important for the process of collagen formation in wound healing. The role of fibroblast cells is very important for the process of collagen formation in wound healing, where interleukin-6 in various theories and studies supports the process of collagen formation.

Keywords: immunohistochemistry; IL-6; keloid formation

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INTRODUCTION

Keloid is a connective tissue disorder of the skin characterized by excessive scar tissue growth beyond the original wound boundaries. The term "keloid" originates from the Greek language, meaning "crab claw," due to its resemblance to a claw-like shape. Despite extensive research on keloids, the precise mechanisms underlying their formation remain incompletely understood. Keloid development is thought to be influenced by multiple factors, including topological factors (areas prone to keloid formation), environmental factors (inflammatory processes), and individual genetic predisposition (Limandjaja et al., 2020; Oei et al., 2021). Epidemiologically, the prevalence of keloids varies worldwide, with the highest incidence observed among African, Asian, and Hispanic populations, ranging from 4.5% to 16%. In Indonesia, a study conducted at the Dermatology and Venereology Clinic of Prof. Dr. Kandou General Hospital, Manado, during the 2017–2018 period reported 93 cases of keloids,

accounting for approximately 1.68% of the total patients (Elazhary et al., 2022; Jannah et al., 2021; Kant et al., 2018).

Various risk factors have been associated with keloid formation. Several studies suggest that individuals with blood type A have a higher tendency to develop keloids spontaneously (Shaheen et al., 2016). Additionally, hypertension has been linked to an increased risk of keloid formation, as vascular damage caused by high blood pressure may enhance the inflammatory response around the wound (Arima et al., 2015). Another contributing factor to keloid formation is the level of melanin pigmentation in the skin, which is suspected to be directly associated with increased collagen production. Keloids also occur more frequently in individuals with elevated immunoglobulin E (IgE) levels, younger age groups (10–30 years), and specific body locations such as the chest, shoulders, and ears (Blalock, 2020; Saheen AA, 2017).

From a pathogenesis perspective, keloids result from disruptions in the wound healing process, primarily due to abnormal inflammatory responses or excessive proliferative phases in dermal fibroblasts. This process leads to the abnormal accumulation of the dermal matrix (Zhang et al., 2024). Recent studies indicate that various inflammatory cells, including macrophages, lymphocytes, and mast cells, play a more significant role in keloid formation than previously thought. These cells produce a range of cytokines that can stimulate fibroblast activity and excessively enhance collagen synthesis, primarily through interleukin (IL) and transforming growth factor (TGF) signaling pathways (Craig et al., 1986).

Although various interleukins have been implicated in the pathogenesis of keloids, interleukin-6 (IL-6) is considered one of the key factors in regulating inflammation and stimulating fibroblast activity (Uitto, 2007). IL-6 has been shown to enhance collagen synthesis and prolong the fibroblast proliferative phase, potentially contributing to uncontrolled keloid growth (Stone et al., 2020). However, despite its frequent mention in the literature as a crucial factor in keloid formation, studies specifically exploring the molecular mechanisms of IL-6 in fibroblast regulation and its interaction with other cytokines within keloid tissue remain limited. Therefore, this study aims to further investigate the role of IL-6 in keloid matrix formation. By understanding how IL-6 contributes to fibroblast proliferation and collagen accumulation, this research is expected to provide new insights into potential therapeutic targets for keloid management. These findings may help bridge existing research gaps regarding the specific mechanisms of IL-6 in keloid pathogenesis and serve as a foundation for the development of cytokine-modulating therapies. Thus, this study aimed to know the role of IL-6 in keloid pathogenesis, as well as a foundation for developing targeted therapies that focus on IL-6 signaling to reduce keloid recurrence in the future.

METHOD

This study utilized formalin-fixed paraffin-embedded (FFPE) keloid tissue samples, which were microscopically diagnosed in the anatomical pathology laboratory of a hospital in Jambi City. All samples were reassessed to confirm their conformity with established keloid diagnostic criteria. Immunohistochemical (IHC) staining was performed at the anatomical pathology laboratory of the Faculty of Medicine and Health Sciences, University of Jambi. The IHC process began with the deparaffinization and rehydration of the FFPE tissue sections. Deparaffinization was conducted by immersing the samples in xylene for three cycles, followed by sequential rehydration in graded ethanol solutions (95%, 90%, 80%, and 70%) for 30 minutes at each concentration. Subsequently, the sections were washed with distilled water and rinsed three times using phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by incubating the samples in normal serum for 30 minutes, followed by three additional washes in PBS. The primary antibody, IL-6 (Santa Cruz

Biotechnology, USA), was applied to the tissue sections and incubated overnight at 4°C. The slides were then washed three times with PBS. Detection was performed using 1,3-diaminobenzidine (DAB) as a chromogen, and the sections were counterstained, dehydrated, cleared, and mounted for microscopic examination. Positive immunostaining was defined by the presence of nuclear staining in fibroblast cells. For each histological section, ten high-power fields (40× magnification) were evaluated. The proportion of IL-6-positive fibroblasts was calculated as a percentage, with a positivity threshold set at 20%. Data collection was carried out using a structured data collection sheet designed to record the immunohistochemical staining results and the socio-demographic data. The sheet included variables such as sample identification number, staining intensity, proportion of IL-6-positive fibroblasts, and any additional histopathological observations. This standardized data collection ensured consistency and accuracy in the recording and analysis of results. Statistical analyses were conducted using SPSS software. Descriptive statistics were used to summarize the data. Differences between groups were analyzed using appropriate statistical tests, with a significance level set at $p < 0.05$. This study was conducted in accordance with the ethical standards approved by the Ethics Committee of the Faculty of Medicine and Health Sciences, University of Jambi.

RESULT

We found 36 cases of keloids, aged between 12 and 42 years, mostly in women (66.7%), most occurred in the trunk area (77.8%), 30 cases recurred (83.3%). We found 24 cases (77.8%) with strong IL-6 immunoexpression in fibroblast cells. There were 3 cases (8.3%) with no immunoexpression. There was a strong correlation between IL-6 immunoexpression with gender ($p = 0.03$), age ($p = 0.04$) and recurrence ($p = 0.02$). Table 1 shows an overview of the clinical characteristics.

Table 1.
Clinical characteristics (n= 36)

Variable	f	%
Age (years)		
10-20	11	30.5
21-30	12	33.3
31-40	10	27.8
41-50	3	8.3
Sex		
Female	24	66.7
Male	12	33.3
Location		
Trunk	28	77.8
Extremities	7	19.4
Head and neck	1	2.72
Size (cm)		
Mean±Std	5.15±2.164	
Median	7.15	
Range (min-max)	5.1 – 10.2	
Type		
Solitary	17	47.2
Multiple	19	52.8
Reccurence		
Yes	30	83.3
No	6	16.7

Table 2 shows the immunohistochemical expression of IL-6, and we found a significant value of IL-6 immunoexpression with age, sex and recurrence. Interleukin-6 is found most often at the age of 21 - 40 years, occurs more often in women and in cases with a history of recurrence

Table 2.
Immunoexpression of Interleukin-6 in Keloid

Variable	IL-6 < 20% (n= 12)	%	IL-6 > 20% (n=24)	%	*p*
Age (years)					
10 - 20	0	0.0	3	100	0.004
21-30	3	25	9	75.0	
31-40	4	40	6	60.0	
41-50	2	66.7	1	33.3	
Sex					
Female	2	16.7	20	83.3	0.03*
Male	8	66.7	4	33.3	
Location					
Trunk	9	32.2	19	67.8	0.534
Extremities	3	42.9	4	57.1	
Head and neck	0	0.0	1	100	
Type					
Solitary	5	29.4	12	70.6	0.765
Multiple	4	21.1	15	78.9	
Recurrence					
Yes	1	3.3	29	96.7	0.002
No	5	83.3	1	1.7	

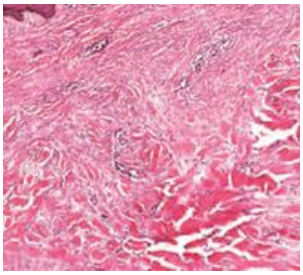
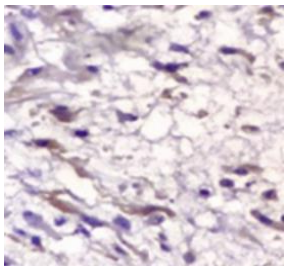
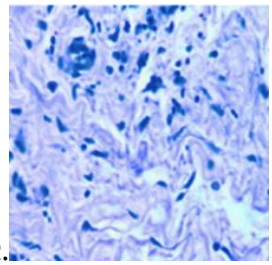
a.  b.  c. 

Fig.1a. Keloid (HE, Olympus 40x) b.Immunoexpression IL-6 >20% (Olympus 40x) c.No immunoexpression IL-6 (Olympus 100x)

DISCUSSION

The findings of this study indicate that IL-6 expression in keloid fibroblasts was high in most cases (77.8%)**,** and this expression was significantly correlated with gender (p=0.03), age (p=0.04), and recurrence (p=0.02)**.** High IL-6 expression was more frequently observed in female patients and in cases with a history of recurrence, particularly in the 21–40 age group. These results are consistent with the study conducted by Johnson et al. (2020), which found that keloid fibroblasts exhibited higher IL-6 expression than normal fibroblasts, contributing to increased fibroblast proliferation and excessive collagen synthesis in keloid tissue (Johnson et al., 2020). Furthermore, research by Xue et al. (2019) also reported that IL-6 plays a central role in pathological scar formation**,** including keloids, through the activation of the JAK/STAT signalling pathway, which promotes excessive extracellular matrix production (Wang et al., 2020; Xue et al., 2000). Additionally, higher IL-6 expression in females has also been reported in previous studies. Horng et al. (2017) indicated that oestrogen can interact with IL-6 and enhance the inflammatory response as well as collagen synthesis in keloid fibroblasts (Horng et al., 2017). Similarly, Yang et al. (2022) supported this finding, showing that keloids are more prevalent in females due to the interaction between hormonal factors and the expression of pro-inflammatory cytokines such as IL-6 (Yang et al., 2022). Interleukin-6 (IL-6) is a 27 kDa glycoprotein consisting of 184 amino acids, and is secreted by inflammatory cells such as macrophages and lymphocytes. This interleukin is thought to be involved in many metabolic processes, such as immune microregulation, IL-6 plays a role in various functions to activate several pathomechanism pathways, for example in the

Ras/Raf/MEK/ERK1/2 pathway to promote tumor cell proliferation. IL-6 -572 GG is significantly associated with an increased risk of keloids. Serum IL-6 is also increased in keloid patients with the GG genotype compared to keloid patients with the CC genotype. IL-6 plays a role in keloid formation (Ghazizadeh, 2007; Quong et al., 2017; Uitto, 2007).

Regarding age, the results of this study show that high IL-6 expression was more common in the 21–40 age group, which is in line with findings from Indramaya et al. (2021)**.** The study reported that younger individuals tend to exhibit a more active wound healing response, with increased production of growth factors and inflammatory cytokines, including IL-6, compared to older individuals (Wardani et al., 2021). This finding may explain why younger individuals are more susceptible to keloid development with higher IL-6 expression**.** From a molecular perspective, IL-6 is recognized as a key cytokine involved in stimulating fibroblast proliferation and increasing collagen synthesis, leading to keloid formation. IL-6 exerts its effects by activating the JAK/STAT signalling pathway**,** which subsequently upregulates the expression of fibrosis-related genes such as COL1A1 (type I collagen) and COL3A1 (type III collagen) (Li et al., 2022). Furthermore, IL-6 also plays a role in recruiting and activating macrophages, which subsequently produce various growth factors such as TGF-β and VEGF**,** contributing to scar formation and increased vascularization in keloid tissue (Liang et al., 2020). This finding aligns with field observations, where keloid lesions with high IL-6 expression also exhibited a greater presence of macrophage infiltration, which may further contribute to keloid recurrence**.**

The correlation between IL-6 expression and keloid recurrence is another key finding of this study. A previous study by Carswell and Borger (2023) found that patients with recurrent keloids exhibited significantly higher serum IL-6 levels than those with non-recurrent keloids (Carswell & Borger, 2023). This supports the notion that IL-6 not only plays a role in the initial development of keloids but also contributes to recurrence mechanisms, potentially by sustaining excessive fibroblast activity (Profyris et al., 2012). Overall, the findings of this study reinforce the hypothesis that IL-6 is a key factor in keloid pathogenesis, influencing fibroblast proliferation, excessive collagen synthesis, and lesion recurrence. Therefore, targeting IL-6 as a therapeutic strategy presents a promising avenue for more effective keloid treatment**.** Some preliminary studies have explored the potential use of IL-6 inhibitors such as tocilizumab, which have shown promise in reducing IL-6 expression and inhibiting keloid growth in experimental models (Lee et al., 2023).

CONCLUSION

The role of fibroblast cells is very important for the process of collagen formation in wound healing, where interleukin-6 in various theories and studies supports the process of collagen formation.

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