Immunoexpression of Interleukin-6 (Il-6) in Keloid

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Abstract. The characteristics of keloid disease are characterized by excessive accumulation of collagen in the dermis and subcutaneous tissue due to damage to skin tissue. This condition causes various complex problems such as loss of self-confidence, low self-esteem, shyness accompanied by obvious physical damage such as contractures, pain, itching and lack of range of motion. Various studies have investigated the genes responsible for this condition including the interleukin (IL)-6 gene. IL-6 is a pleiotropic cytokine with multiple biological functions. Naturally, these cytokines are produced locally upon intense and sustained damage and initiate transcriptional provocative reactions via the IL-6 alpha receptor. The purpose of this study was to analyze Interleukin-6 immunoexpression in keloids. This research is an observational study with a cross sectional research design. Samples were stored in formalin-fixed and paraffin-embedded (FFPE) material which was diagnosed histopathologically as a keloid disease. The research variable to be examined is IL-6 immunoexpression. The examination technique used is immunohistochemical examination. There were 31 cases of keloids, aged between 16 and 47 years, mostly in women (67.7%), occurring in the trunk area (70.9%) 18 cases of recurrence (58%). We found 24 cases (77.4%) with strong IL-6 immunoexpression in fibroblast cells. There were 7 cases (22.6%) with weak immunoexpression. There was a strong correlation between IL-6 immunoexpression and age (p=0.04) and recurrence (p=0.02). There is no significant relationship with gender. Fibroblasts produce collagen and fibronectin which are necessary for wound re-epithelialization. Remodeling of these deposits and matrices is a continuous process and is influenced by various growth factors. IL-6 cytokine is produced more by dermal than superficial fibroblasts, this is what determines the difference between deep and superficial wound healing. This can be considered as regulatory factor IL-6 in keloid disease.

Keywords: Immunoexpression · Interleukin-6 · Keloid

1 Introduction

Keloids are benign proliferative dermal collagen growths and are characterized by excessive accumulation of extracellular matrix components, particularly type 1 collagen, fibronectin and proteoglycans [1]. Clinical manifestations appear as erythematous

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lesions that grow larger than skin lesions and rarely regress over time. The characteristics of keloid disease are characterized by excessive accumulation of collagen in the dermis and subcutaneous tissue due to damage to skin tissue. This condition causes various complex problems such as loss of self-confidence, low self-esteem, embarrassment accompanied by obvious physical damage such as contractures, pain, itching and lack of range of motion. In addition, the treatment modalities for this disease are still unsatisfactory and have a high recurrence rate [2]. Several genes are involved in this condition such as human leukocyte antigen (hla) alleles, mitogen-activated protein kinase (mapk), transforming growth factor (tgf)-β, interleukin (il)-6 and plasminogen activator inhibitor[3, 4] various studies regarding the genes responsible for this condition include the interleukin (il)-6 gene. Il-6 is a pleiotropic cytokine with multiple biological functions. Naturally, these cytokines are produced locally upon intense and sustained damage and initiate transcriptional provocative reactions via the il-6 alpha receptor. In the normal process, expression of il-6 will decrease significantly during the remodeling phase [5]. Increased expression and production of il-6 in keloid patients has been reported, indicating changes in autocrine il-6 upregulation in keloid fibroblasts [6] the purpose of this study was to analyze immunoexpression interleukin-6 in keloids.

2 Methods

This research is an observational study with a cross sectional research design. All cases were diagnosed histopathologically at the pathology laboratory in Jambi province. Samples were stored in formalin-fixed and paraffin-embedded (FFPE) materials. Immunohistochemical staining (IHC) was carried out in the biomedical laboratory of the Faculty of Medicine and Health Sciences, Jambi University. The initial step is deparaffinization and rehydration of the sample for 30 minutes. Paraffin block samples in 3-phase xylol liquid. Then the rehydration process was carried out with an alcohol solution (95%, 90%, 80%, and 70%) for 30 minutes for each liquid. Then the samples were washed using distilled water and rinsed with phosphate buffer saline (pbs) three times. The specimens were then incubated in normal serum for 30 minutes and rinsed with PBS solution 3 times, incubated with IL-6 antibody (Santa Cruz Biotechnology, USA) in the refrigerator overnight, then rinsed again with PBS 3 times.

Then the samples were stained with 1,3-diaminobenzidine, rinsed with distilled water followed by dehydration, clearing, and mounting. Immunostaining is interpreted as positive when staining of tumor nuclei is detected. The number of positive cells was evaluated in 10 high power fields (40x) for each histology section and calculated as the percentage of tumor positive cells where the cut off was 20%. Results can be said to be significant if $p < 0.05$. Statistical analysis used SPSS software for windows (V.24.0). This research was approved by the Ethics Committee of the Faculty of Medicine and Health Sciences, Jambi University. A/762UN21.7/PT/2022.

3 Result

There were 31 cases of keloids, aged between 16 and 47 years, mostly in women (67.7%), occurring in the trunk area (70.9%), 18 cases of recurrence (58%) (Table 1). We found 24 cases (77.4%) with strong IL-6 immunoexpression in fibroblast cells. There were 7

Tabel 1. Clinical characteristics

Variable	N = 31
Age (years)	
10–20	4 (12.9%
21–30 31–40 41–50	10 (32.3%) 9(29%) 8 (25.8%)
Sex Female Male **Location**	21(67.7%) 10 (32.3%)
Trunk	22(70.9%)
Extremitas Head and neck	8(25.9%) 1(3.2%)
Size (cm)	
Mean±Std	7.65±2.765
Median	8.05
Range (min-max)	5.6 – 13.6
Type	
Solitary	15(48.4%)
Multiple	16(51.6%)
Recurrence	
Yes	10(32.3%)
No	21(67.7%)

cases (22.6%) with weak immunoexpression. There was a strong correlation between IL-6 immunoexpression and age (p=0.04) and recurrence (p=0.02). There is no significant relationship with gender (Table 2). Fibroblasts produce collagen and fibronectin which are necessary for wound re-epithelialization. Remodeling of these deposits and matrices is a continuous process and is influenced by various growth factors. The cytokine IL-6 is produced more in dermal fibroblasts than superficial fibroblasts, this makes the wound healing process different between superficial and deep wounds immunoexpression was found in more than 20% of the masses in cases of recurrent keloids (Fig. 1).

4 Discussion

The wound healing process consists of several overlapping phases, namely hemostasis, inflammation, proliferation and remodeling of the extracellular matrix which is arranged in great detail to prevent damage and damaged epidermal barrier can be repaired quickly. The inflammatory response is generated by a series of process interactions between

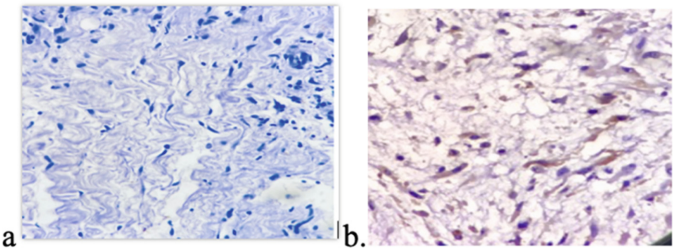

Fig. 1. Immunoexpression of IL-6 a. No immunoexpression b. Immunoexpression > 20%

Table 2. Immunoexpression Interluekin-6 in Keloid

IL-6	**IL-6 < 20% (n = 7)**	**IL-6 > 20% (n = 24)**	***P***
Age	0 (0.0%)	4 (16.6%)	**0.04***
10–20	1(14.28%)	9 (37.5%)	
21–30	2 (28.6%)	7 (29.16%)	
31–40	4 (57.1%)	4 (16.6%)	
41–50			
Sex			
Female	1(15.4%)	20(11.7%)	**0.567**
Male	6(84.6%)	4 (88,3%)	
Location	3(42.9%)	19(79.2%)	**0.534**
Trunk	4(57.1%)	4(16.6%)	**0.765**
Extremitas	0 (0.0%)	1(4,2%)	**0.02***
Head and neck	3(42.9%)	12 (50%)	
Type	4 (57.1%)	12(50%)	
Solitary	1 (15.4%)	20 (83.3%)	
Multiple	6 (84.6%)	4(16.6%)	
Recurrence			
Yes			
No			

resident and systemic immune cells and specific tissue cell types as the primary triggers of post-injury scarring.[7, 8] To explain our goal, we selected 30 samples from the stored material.. In line with Abdul Allah et all, There was no significant difference between the age group (16–47 years) with other studies[9] In line with Ojeh et al., this study showed that keloids are mostly in women, although men and women have the same risk, this can be because women have a tendency to perform cosmetic procedures such as ear piercing[10]. Omo-Dare studied 34 families in the Nigerian population and concluded that there is a role for inherited genetics. Several studies state that there are several HLA alleles, mitogen-activated protein kinase (MAPK), transforming growth factor (TGF)-β, interluekins (IL)-6, and plasminogen activator inhibitor (PAI)-1 which play a role in keloid formation. The immune response has an important role in the initial

wound healing process and the final wound formation, the longer the inflammatory process, the worse the scar tissue results. Target cells (fibroblasts) and effector cells help the immune cell complex traverse the wound microenvironment. Keloid fibroblast cells are associated with nuclear factor (NF)-kB signaling, resulting in upregulation of inflammatory mediators (IL-1α, IL-1β, IL-6) and TNF-α.(3) IL-6 and IL-8 are expressed immediately after skin breakdown and will continue to be expressed at high levels for several days. These cytokines attract and activate inflammatory cells and vascularize the keloid scar, allowing it to overgrow [13–15].

Several studies have shown that IL-6 is a 27 kDa glycoprotein consisting of 184 amino acids, and is secreted by inflammatory cells (eg lymphocytes and macrophages). It has been confirmed that IL-6 is involved in many metabolic processes, such as regulation of the immune microenvironment. IL-6 plays a role in various biological functions by activating several signaling pathways. For example, activating 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 was increased in keloid patients with GG genotype compared to keloid patients with CC genotype. They concluded that IL-6 plays a role in keloid scar formation[16, 17].

Similar to a study conducted by Limin Luo et al., their study showed that mRNA concentration and adiponectin expression were low, TGF-β1, CTGF, IL-6 and TNF-α levels were high in patients with keloids compared to patients without keloids. Keloids. They ascribed a decrease in serum adiponectin to be related to the development and formation of keloids [18]. In our study it was shown that increased IL-6 in patients with keloids, IL-6 is involved in many metabolic processes. As we know dermal fibroblasts produce more IL-6 than superficial fibroblasts, this process is related to excess proliferation and reduces dermal fibroblast apoptosis, so that IL-6 can be considered as a causative factor for keloid disease. We recommend further study on a large sample size.

5 Conclusion

This can be considered as regulatory factor IL-6 in keloid disease. There is no significant relationship with gender. Fibroblasts produce collagen and fibronectin which are necessary for wound re-epithelialization. Remodeling of these deposits and matrices is a continuous process and is influenced by various growth factors. IL-6 cytokine is produced more by dermal than superficial fibroblasts, this is what determines the difference between deep and superficial wound healing.

Author Contributor

FZ: Data collection, article preparation, section writing, Figs drawing; FY: Writing section; EAU: Writing section, modification; HY: Writing section, preparation of articles.

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