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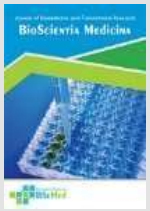

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Combination Therapy of Topical Antioxidant Gel and Platelet-Rich Plasma (PRP) in Pyoderma Gangrenosum Ulcer: A Case Report

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A B S T R A C T

Background: Pyoderma gangrenosum (PG) is a rare necrotic ulcerative skin disease, often associated with an underlying systemic condition. Bacterial coinfection in PG can worsen the course of the disease and slow healing. **Case presentation:** We report the case of a 25-year-old woman with PG of her left leg complicated by *Pseudomonas aeruginosa* co-infection. The patient had a history of poorly treated psoriasis. The diagnosis is made based on disease history, physical examination, microbiological examination, and histopathological examination. The patient was treated with oral levofloxacin and topical combination therapy of astaxanthin and platelet-rich plasma (PRP) with wound debridement. Significant clinical improvement was achieved within six weeks. **Conclusion:** PG with bacterial coinfection requires appropriate diagnosis and treatment to achieve optimal results. Topical therapy combining astaxanthin and PRP with wound debridement proved effective in this case.

1. Introduction

Pyoderma gangrenosum (PG) is a neutrophilic dermatosis characterized by erythematous nodules or sterile pustules that develop into painful violaceous ulcers, particularly on the lower legs. Pyoderma gangrenosum is commonly associated with systemic diseases such as inflammatory bowel disease (IBD), acute leukemia, pyogenic arthritis pyoderma gangrenosum and acne syndrome (PAPA syndrome), and sweet syndrome or Adamantiades-Behçet disease.[1] The goal of PG management is to reduce the inflammation process and promote wound healing to achieve appropriate treatment regarding the underlying disease and severity of the disease. Wound care is essential management to avoid the risk of secondary infection and the occurrence of new lesions due to friction as known by pathergy.[2]

Skin wound healing is a unique interaction of several cells, growth factors, and cytokines. Recently oxidative processes are also involved in wound pathogenesis. Excess production of reactive oxygen species (ROS), a major oxidized product, contributes to cell damage and dysregulates wound healing process.[3] Recent studies evaluate the effectiveness of antioxidant agents in wound care management including astaxanthin, a xantophyll carotenoid that is

contained in natural marine, yeast, salmon, trout, krill, shrimp, and crayfish. It has numerous biological activities such as antioxidant, anti-lipid peroxidation, antiinflammation, and immune modulation.[4] Rofiq et al conducted a study to evaluate the effectiveness of 5% astaxanthin gel in a second-degree burn animal model. They reported that 5% astaxanthin gel induces the angiogenesis and granulation tissue formation process particularly in the second phase of wound healing.[5]

Recent optional wound care management that is widely used is platelet-rich plasma (PRP), an autologous plasma derivate that contains abundant growth factors and cytokines mainly in alpha granules of platelets. In chronic diseases such as diabetic ulcers, overproduction of oxidative stress leads imbalance of pro-inflammatory and anti-inflammatory cytokines which causes wound healing disturbance. Platelet-rich plasma contains high levels of growth factors and cytokines that maintain ROS concentration as well as reduce wound recovery time.[6] Several clinical studies have observed the effectiveness of PRP-based therapy. Babei et al reported a significant granulation formation and early wound closure in 150 patients with diabetic ulcers that treated with topical PRP.[7] Meanwhile Cieslik-Bielecka et al also reported clinical improvement and faster wound healing process after topical PRP application in human immunodeficiency virus (HIV) infected patients who suffering from crural ulcer.[8] Hence, we reported a case of pyoderma gangrenosum ulcer treated by combining astaxanthin gel with PRP so that can be used as an alternative treatment, especially in chronic recalcitrant skin ulcer cases.

2. Case Presentation

A 25-year-old female was admitted to the dermatovenereology outpatient clinic of Dr. Moewardi Regional General Hospital, Surakarta, Indonesia due to a chronic ulcer on her left leg. The first lesion appeared 5 years prior as a red pustule which ruptured easily and became an extensive painful ulcer. She was seeking medication and got systemic methylprednisolone followed by wound debridement, unfortunately no clinical improvement. The patient had suffered from psoriasis but was untreated well. On clinical examination, the patient presented multiple ulcers of various sizes with a violaceus undermined edge and purulent necrotic materials (Figure 1A).

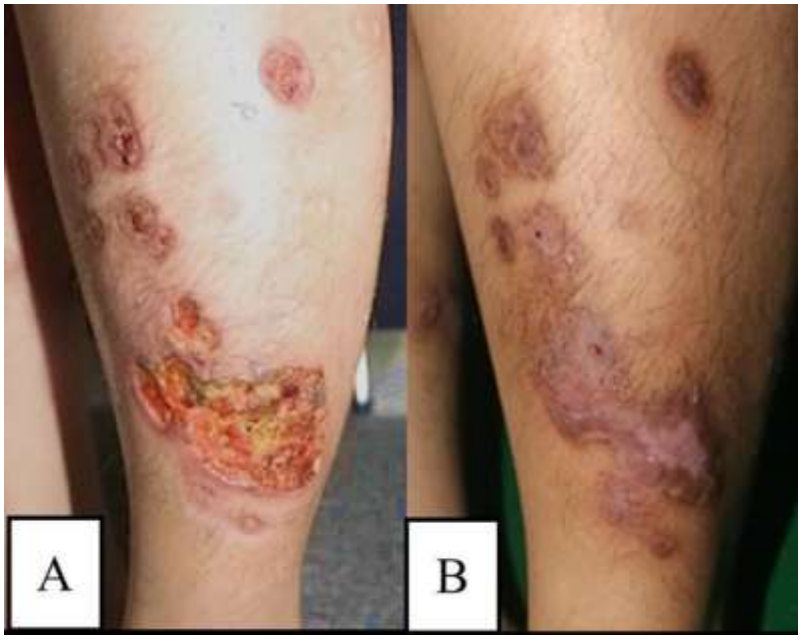

Figure 1. The clinical manifestations of patients before (A) and after treatment (B).

Swab cultures of the leg ulcer showed growth of *Pseudomonas aeruginosa* which was levofloxacin sensitized. Based on histopathological features hematoxylin and eosin staining demonstrated intense dermal neutrophilic infiltration and was followed by septal panniculitis (Figure 2). According to the history of illness, physical examination, microbiological examinations, and histopathological findings, the patient was diagnosed with pyoderma gangrenosum with *P. aeruginosa* co-infection. The skin co-infection was managed with oral levofloxacin 500 mg daily for 7 days, while the skin ulcer was treated with the mixture of topical astaxanthin gel (30 grams) with PRP (1.2 mL) and followed by wound debridement applied twice a day. The process of making PRP is carried out by 10-20 mL of the patient's venous blood is taken using a vacuum tube containing an anticoagulant such as sodium citrate or acid citrate dextrose (ACD); Make sure the vacuum tube is completely filled with avoid red blood cell hemolysis; Mix blood with anticoagulant carefully to prevent blood clots; Initial centrifugation: Performed at low speed (approximately 150-300 g) for 10-15 minutes to separate red blood cells from plasma and buffy coat (the layer containing white blood cells and platelets); Second centrifugation: Performed at a higher speed (approximately 1000-1500 g) for 15-20 minutes to separate the platelet-rich plasma (PRP) from the buffy coat. The PRP is located at the top of the tube, on top of the buffy coat layer; PRP is collected carefully using a sterile pipette or syringe; Avoid taking a buffy coat because it can cause contamination of leukocytes and red blood cells in the PRP; PRP can be stored in a sterile container at 4°C for 24-48 hours. The clinical improvement was achieved in the sixth week of therapy as cribriform appearance (Figure 1B).

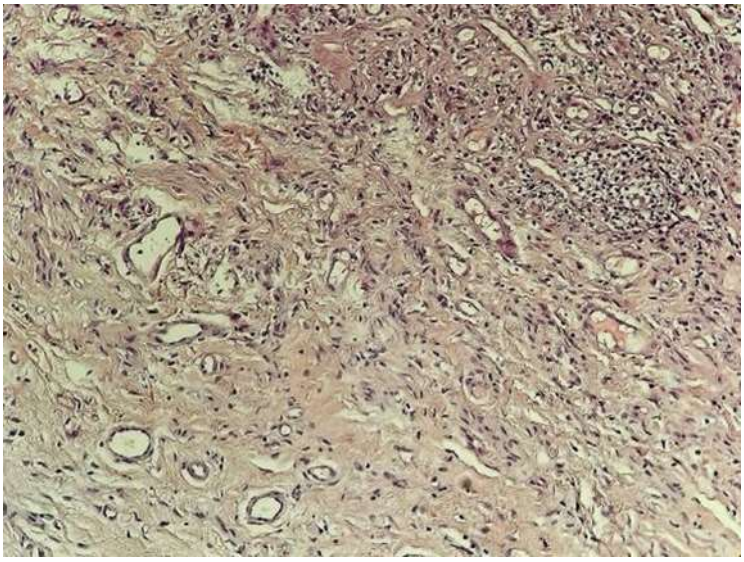

Figure 2. The histopathological examination showed intense dermal neutrophilic infiltration followed by septal panniculitis (H&E stain, 40x).

3. Discussion

Pyoderma gangrenosum is an uncommon neutrophilic inflammatory skin condition that progressively develops into a chronic ulcer. It is estimated to affect up to 10 cases per million people per year with up to 3% of chronic leg ulcer cases. Epidemiological studies stated that PG mostly affected the second to sixth decades of life, with a possible female predominance. The majority of reported PG cases are associated with underlying systemic diseases and genetic mutations, meanwhile, hematologic malignancy has been reported in up to 7% of cases.[2] The cutaneous manifestation of PG commonly presents as the classic ulcerative variant which is characterized by erythematous nodules or sterile pustules that gradually develop into painful

violaceous chronic recurrent ulcers with the most common site being the legs.[1] The histologic changes are nonspecific depending on the clinical variant of PG (ulcerative, bullous, pustular, or vegetative), the timing, and the site of the biopsy. The main purpose of histopathological examination is to exclude other diagnoses. Classical ulcerative PG commonly shows a sterile neutrophilic infiltration within the dermis, abscess formation, and neutrophilic pustules. Vasculitis has been observed in some cases due to the ulcerations themselves instead of a characteristic essential finding. When the vasculitis is seen on histology, care must be taken to rule out the true causes of both infection and vasculitis.[9]

In our case, the patient was a twenty-five-year-old female with chronic painful skin ulcers on her left leg. She also suffered from uncontrolled psoriasis. Based on physical examination showed multiple ulcers with purplish-based margins followed by purulent necrotic materials. The histopathological features obtained a high density of neutrophilic dermal infiltration with septal panniculitis. According to those findings we diagnosed the patient with PG. The mainstays of management are systemic immunosuppressive agents that are combined with appropriate local and topical therapy. Moreover, the management approach of PG is to be considered according to associated conditions and the severity of the disease.[2] Systemic immunosuppressive therapy is the first line of therapy for more severe or extensive conditions, while mild PG with small lesions can be treated with topical therapy. Topical corticosteroid or triamcinolone injection around the ulcer's edge can be used to treat the ulcer, another responsive treatment has been reported by using topical tacrolimus.[10] In this case, the patient had already been given systemic methylprednisolone and wound debridement previously but no significant improvement was achieved. Based on clinical assessment, the severity of PG in our case was mild so we decided to treat the skin ulcer topically by using a mixture of astaxanthin gel with PRP. Meanwhile, the skin co-infection condition was treated with oral antibiotics in the short-term period.

The exact pathophysiology of PG is not fully understood, although several hypotheses have been proposed. Aberrant cytokine production is responsible for the increased incidence of PG, where elevated levels of neutrophil chemokines such as IL-8, chemokine C-C ligand-3 (CCL)-3, and CCL-5 were detected in PG lesions. Those conditions increase the tendency to form neutrophil extracellular traps (NETs), where aged neutrophils will produce higher levels of ROS by upregulation of chemokine receptor CXCR4 expression on the cell surface.[11,12] Furthermore, tumor necrosis factor-alpha (TNF-α) plays an important role in the production of proinflammatory cytokines, including interleukin 1 (IL-1), IL-6, IL-8, and interferon-gamma (IFN- γ) will stimulate the superoxide production. The abundant of IL-8 level also contributes to the necrotic skin ulcer formation as well inflammation process.[2]

Based on the recent pathogenesis of PG, we decided to use astaxanthin gel as an antioxidant agent to treat the skin ulcer. Astaxanthin reduces oxidative stress and protects against tissue and organ damage by influencing the activity of antioxidant enzymes, beside that the inhibition effects on polymorphonuclear leukocyte infiltration and cytokine release will decrease the inflammatory process.[13] Moreover, the anti-inflammatory properties of astaxanthin inhibited the specific inflammation-associated signaling molecules including nuclear factor kappa B (NF-KB) and PI3K/Akt which might lead to the secondary release of inflammatory cytokines.[14] Astaxanthin is a carotenoid found naturally in the algae *Haematococcus pluvialis* and several other marine organisms. Astaxanthin has much stronger antioxidant capabilities than vitamins E and C, making it an effective antioxidant agent in combating oxidative stress. Oxidative stress is an imbalance between free radicals and antioxidants in the body, which can cause cell and tissue damage. Astaxanthin can neutralize free radicals and reduce oxidative stress, thereby protecting healthy skin cells from damage. Astaxanthin can increase the proliferation of fibroblast and keratinocyte cells, which are important

for the wound healing process. Astaxanthin can increase the formation of new blood vessels, which are important for supplying oxygen and nutrients to healing wounds. Astaxanthin can inhibit the activity of pro-inflammatory enzymes and reduce the production of inflammatory cytokines, thereby helping to reduce inflammation in wounds. Astaxanthin can increase the production of collagen, the main structural protein in the skin, which is important for skin strength and elasticity. Several clinical studies have shown that astaxanthin may be beneficial in the treatment of pyoderma gangrenosum wounds. A study of 20 patients with PG found that taking oral astaxanthin 20 mg twice daily for 8 weeks significantly reduced ulcer size, pain, and inflammation compared with the placebo group. A study of 10 patients with PG found that the use of topical astaxanthin 1% twice daily for 4 weeks significantly improved wound healing and reduced inflammation compared with the placebo group. Astaxanthin is a powerful antioxidant with anti-inflammatory properties that may be beneficial in the treatment of pyoderma gangrenosum wounds. Clinical evidence shows that astaxanthin may help reduce ulcer size, pain, and inflammation, and improve wound healing.[12-15]

Over the last few decades, PRP-based therapy in wound care management was highly raising. A case report done by Naveed et al revealed the effectiveness of PRP in PG patients which not respond to conventional therapies, besides that Alvarez-Lopez et al also reported a successful PRP-based treatment in refractory PG cases.[15,16] Anti-inflammatory effect of PRP by decreasing the inflammatory cytokines such as IL-17 and IL-1β, moreover it promotes angiogenesis of wound tissue by promoting vascular endothelial growth factor (VEGF) secretion and CD31 expression. Platelet-rich plasma induces the expression of insulin growth factor 1 (IGF-1) which is an important growth factor for epidermal cell proliferation particularly in wound tissue.[17] Pyoderma gangrenosum (PG) is a rare necrotic ulcerative skin disease characterized by sterile pustules that rapidly develop into ulcers with raised edges and purple-blue necrosis. PG is often associated with underlying systemic conditions, such as ulcerative colitis, Crohn's disease, rheumatoid arthritis, and myeloproliferative disorders. Treatment of PG generally focuses on controlling the underlying systemic condition and managing local inflammation. Platelet-rich plasma (PRP) is increasingly popular as an adjunct therapy for PG because it is rich in growth factors that can promote wound healing. PRP is a blood plasma concentrate enriched with platelets. Platelets contain many growth factors and proteins involved in various biological processes, including wound healing. Platelet-derived growth factor (PDGF) is a powerful growth factor that stimulates the proliferation and migration of fibroblasts, cells important for the synthesis of collagen and extracellular matrix. PDGF also increases angiogenesis, the formation of new blood vessels, which is important for supplying oxygen and nutrients to healing wounds. Transforming growth factor-beta (TGF-β) is a growth factor involved in a variety of biological processes, including wound healing, inflammation, and immunosuppression. TGF-β stimulates the synthesis of collagen and extracellular matrix, and also reduces inflammation, and improves immunosuppression in wounds. Vascular endothelial growth factor (VEGF) is a growth factor that is important for angiogenesis. VEGF stimulates the formation of new blood vessels, which are important for supplying oxygen and nutrients to healing wounds. Epidermal growth factor (EGF) is a growth factor that stimulates the proliferation and migration of keratinocytes, cells that are important for the regeneration of the epidermis. EGF also increases angiogenesis and reduces inflammation in wounds. Insulin-like growth factor-1 (IGF-1) is a growth factor that stimulates the proliferation and migration of various types of cells, including fibroblasts, keratinocytes, and smooth muscle cells. IGF-1 also increases collagen and extracellular matrix synthesis. PRP works by releasing growth factors contained in platelets into the wound. These growth factors then interact with various types of cells in the wound, stimulating proliferation, migration, and protein synthesis. This leads to increased angiogenesis,

collagen and extracellular matrix synthesis, and reduced inflammation, all of which are important for wound healing. A study of 15 patients with PG found that twice daily use of topical PRP for 4 weeks significantly improved wound healing and reduced inflammation compared with the placebo group. A study of 10 patients with PG found that subcutaneous and topical use of PRP twice weekly for 4 weeks significantly reduced ulcer size and pain compared with the placebo group. Further research is needed to confirm the effectiveness of PRP in the treatment of pyoderma gangrenosum wounds and to determine the optimal dose and route of administration. PRP is a promising therapy for the treatment of pyoderma gangrenosum wounds. The growth factors in PRP can stimulate various biological processes that are important for wound healing, such as angiogenesis, synthesis of collagen and extracellular matrix, and reduction of inflammation.[15-17]

The combination of astaxanthin and PRP can provide a synergistic effect in healing Pyoderma Gangrenosum wounds. Astaxanthin may enhance the antioxidant and anti-inflammatory effects of PRP, while PRP may help deliver astaxanthin to the wound site more effectively. The combination of astaxanthin and PRP can increase the proliferation of fibroblast and keratinocyte cells more significantly compared to astaxanthin or PRP alone. The combination of astaxanthin and PRP can increase the formation of new blood vessels more significantly compared to astaxanthin or PRP alone. The combination of astaxanthin and PRP can inhibit the activity of pro-inflammatory enzymes and reduce the production of inflammatory cytokines more significantly compared to astaxanthin or PRP alone. The combination of astaxanthin and PRP can increase collagen production more significantly compared to astaxanthin or PRP alone. The combination of astaxanthin and PRP has the potential to be an effective therapy for pyoderma gangrenosum. In this case, we combined topical astaxanthin gel as an antioxidant property with PRP to treat a recurrent PG case. The beneficial effects of this mixture are the reduction of oxidative stress in wound tissue and wound closure acceleration. There are no adverse events have been reported during the therapy and clinical improvement was achieved in the sixth week characterized by cribriform appearance.[18-20]

4. Conclusion

Chronic skin ulcers caused by PG are commonly hard to treat, so appropriate treatment is needed especially for wound care management. Several factors may contribute to the pathogenesis of PG including oxidative stress properties. The mixture of topical astaxanthin gel with PRP showed a beneficial effect in treating recurrent PG ulcers due to antioxidant activity and tissue repair by growth factor-mediated.

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