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Potential Delayed Healing in Wounds with Scabies Comorbidity: A Review about Immunological State and Oxidative Stress in Scabies Infestation

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ABSTRACT

Scabies is a highly contagious skin disease caused by the human itch mite Sarcoptes scabiei var. hominid. Having scabies infestation coexisting in the same human body with a wound potentially lead to delayed wound healing. We constituted this literature review discussing wound healing in relation woth immunological state and oxidative stress in scabies infestation using information from literatures obtained from Google Scholar and PubMed.

The immune response to scabies infection involves multiple layers of defense mechanisms, including the skin's defense mechanisms, T-cells, keratinocytes, and cytokines. Scabies mites can trigger upregulation of pro-inflammatory cytokines interleukins-1α (IL-1α) and interleukins-1β (IL-1β), which play a critical role in the early phases of wound healing by promoting the recruitment of immune cells and stimulating the production of growth factors and extracellular matrix proteins. Scabies mite infestation also triggers a sustained release of reactive oxygen species (ROS) from neutrophils which later becomes an oxidative stress leading to delayed healing and chronic inflammation. Topical treatment for scabies usually involves pesticide substances which, despite its therapeutic effect, have side effect of triggering ROS production. Comprehending the immunological state and oxidative stress in scabies infestation is essential in planning proper management for every wound with scabies comorbidity.

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INTRODUCTION

Scabies is a skin disease first discovered by Ferdinand von Hebra and in 1687 described by Giovanni Bonomo as an infestation of by the mite Sarcoptes scabiei var. hominid, the anthropod phylum of the Sarcoptidae family. Scabies is a contagious tropical skin disease that triggers severe itching and discomfort and its name comes from the Latin word *scabere* which means to scratch. It is a highly prevalent skin disease that affects humans and animals, and often overlooked due to its non-life-threatening nature. However, the disease can become chronic and cause dangerous complications, including secondary infections with Group A Streptococcus and Staphylococcus aureus bacteria.[1,2]

Scabies is more common in populations with low socio-economic status, poor hygiene, and high residential density like dormitories, orphanages, or prisons.[3] It causes intense itchiness, and the lesions caused by Sarcoptes scabiei take a long time to heal, causing discomfort and disruption to patients' lives.[1] Scabies is officially named a neglected tropical disease in 2017, as it affects about 300 millions of global population annually, from all ages, races, and socio-economic levels, with sensory-deficient and immune-compromised patients, children and young adults, and the elderly as the groups of higher risk of infection. Scabies can also lead to impetigo, severe bacterial infections, and post-infectious complications. After almost 50 years, research on scabies control has shown great promise, especially on highly endemic islands. Further understanding of the disease mechanism is necessary to develop effective treatments and control strategies,[4] including an understanding of the immunological status and healing process of a wound in patients with comorbid scabies that we review in this article.

Figure 1. Four cardinals signs of scabies.

Clinical features of scabies

Scabies has 4 cardinal signs: (1) intense nocturnal itching, (2) tunnels in the skin, (3) attacking a group of people, and (4) the presence of mites and their eggs in the primary lesion or in the tunnel (**see Figure 1**). The primary lesion is a small papule or vesicle resulting from mite intrusion and tunneling, with secondary lesions taking the form of papules, pustules, vesicles, and bullae. Tertiary lesions may also develop, such as excoriation and pyoderma. Scabies transmission occurs through skin-to-skin contact or contact with contaminated items. Diagnosis can be established by finding two of the four main signs, and investigations include skin scrapings, mite collection, or biopsy. Scabies has several differential diagnoses --atopic dermatitis, papular urticaria, contact dermatitis, prurigo, and other pruritic diseases, and treatment typically involves topical or systemic medications.[1]

Immunological state during scabies infestation

Scabies infection triggers a complex immune response in the human body, with various layers of defence mechanisms coming into play. The skin's defence mechanisms, which form the first line of defence, play a crucial role in detecting antigen or allergen exposure and initiating immune cell response. T-cells, which are essential in recognizing antigens and inducing cytokine production, play a central role in activating and regulating the immune response during scabies infection. Keratinocytes, which produce pro-inflammatory cytokines like interleukins-1 (IL-1), interleukins-6 (IL-6), interleukins-8 (IL-8), and tumor necrosis factor α (TNF-α), also contribute to the immune response by producing immunomodulatory cytokines like interleukins-10 (IL-10) and interleukins-12 (IL-12). Recent studies suggest that scabies mite proteins play a crucial role in modulating cytokine and chemokine secretion, and the expression of adhesion molecules from skin cells like fibroblasts, keratinocytes, and endothelial cells. In vitro studies have shown that Sarcoptes scabiei var. canis can upregulate interleukins-1α (IL-1α) and interleukins-1β (IL-1β) secretion in human skin-equivalent cell culture supernatants. It showed the extract of the mite downregulated the secretion of interleukin-1 receptor antagonists (IL-1RA) and interleukin-8 receptor antagonists (IL-8RA), and stimulate the secretion of interleukins-6 (IL-6) and vascular endothelial cell growth factor (VEGF) in normal human epidermal keratinocytes. It also showed the upregulation of interleukins-6 (IL-6), interleukins-8 (IL-8), granulocyte colony-stimulating factor (GCSF), and vascular endothelial cell growth factor (VEGF) in normal human dermal fibroblasts.[5,6]

One type of scabies, crusted (Norwegian) scabies, has its clinical presentation quite similar to psoriasis, with erythematous scaly papules and plaque formation due to abnormal keratinocyte infiltration and hyperproliferation of inflammatory cells into the epidermis and dermis (**see Figure 2**). Studies suggest that psoriasis-like development starts and maintained by overexpression of T-helper 1 (Th1) cytokine complexes like interleukins-2 (IL-2), interleukins-6 (IL-6), interleukins-8 (IL-8), interferons-c (IFN-c), and tumor necrosis factor α (TNF-α). A preliminary study of cytokine production from peripheral blood mononuclear cell (PBMC) samples of patients with crusted scabies showed some interesting findings among others: that there was significant increase in interleukins-4 (IL-4), which potentially stimulates keratinocyte proliferation, that epidermal cells had interleukins-4 (IL-4) receptors, and that interleukin-4 receptor (IL-4R) expression increased in psoriasis-like process. Microarray analysis of peripheral blood

mononuclear cell (PBMC) samples obtained from recurrent scabies patients revealed significant upregulation of amphiregulin and epiregulin during times of severe disease.[5]

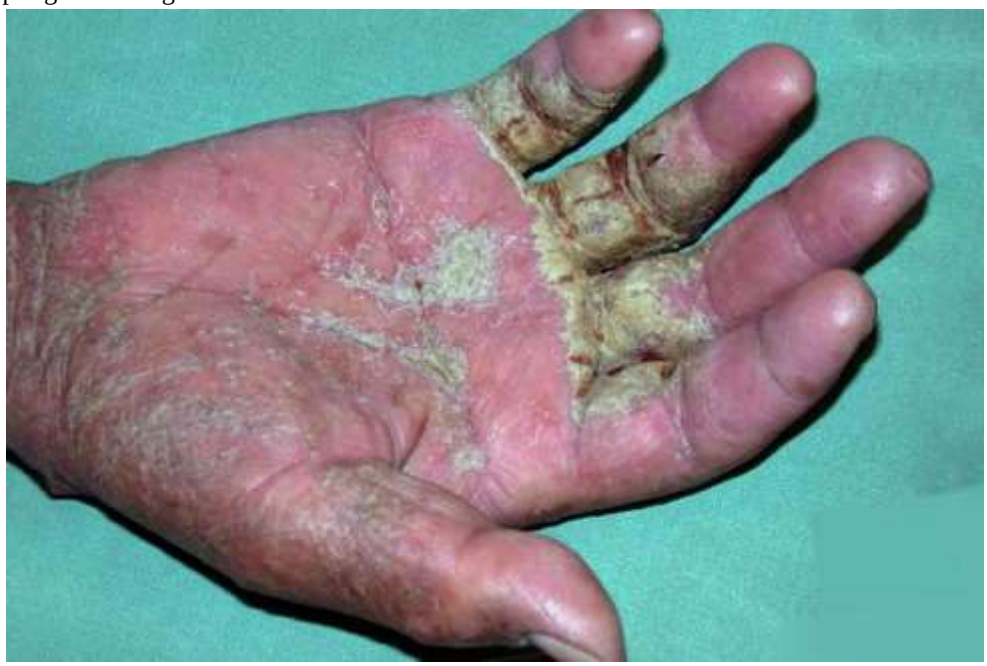

Figure 2. Crusted (Norwegian) scabies.

Overall, the immunological defense mechanisms of scabies patients are complex and involve multiple layers of immune cell response and cytokine production. Further research into the mechanisms involved in scabies infection may lead to new therapeutic strategies.

Effect of scabies comorbidity and inflammation phase of wound healing

Wounds are a breakdown of the skin that can be caused by surgery, trauma, or infection.[7] When scabies infestation and a wound coexist in the same human body, they can have a significant impact on the wound healing process and potentially lead to delayed wound healing.

The cellular processes involved in wound healing are complex and involve various cell types and signaling molecules. During the inflammatory phase of wound healing, immune cells, such as neutrophils and macrophages, migrate to the site of injury and release cytokines, such as interleukins-1 (IL-1) and tumor necrosis factor α (TNF-α), to clear the wound of bacteria and debris. These cytokines also stimulate the proliferation and migration of fibroblasts, which are responsible for producing extracellular matrix (ECM) and collagen to form new tissue.[8,9]

Scabies infection can disrupt the normal wound healing process through several mechanisms. The intense itching associated with scabies can cause scratching and at any given time the scratching activity may in contact and further damage the wound site, leading to a more extensive tissue damage and prolonged healing process. Furthermore, scabies mites and their faeces can also trigger an inflammatory response in the skin, and such immune response lead to an overproduction of cytokines, which certainly interfere with the normal wound healing process.

As we explained earlier in this article, scabies mites can trigger upregulation of pro-inflammatory cytokines interleukins-1α (IL-1α) and interleukins-1β (IL-1β) which play a critical role in the early phases of wound healing by promoting the recruitment of immune cells and stimulating the production of growth factors and extracellular matrix proteins.[6] Those interleukins bind to the interleukin-1 (IL-1) receptor on the surface of target cells and activate a signalling pathway that leads to the expression of inflammatory genes, including cytokines, chemokines, and adhesion molecules. While the early production of interleukins-1α (IL-1α) and interleukins-1β (IL-1β) is necessary for initiating the wound healing process, sustained upregulation of these cytokines can lead to chronic inflammation and delayed healing (**see Figure 3**).[6,10,11]

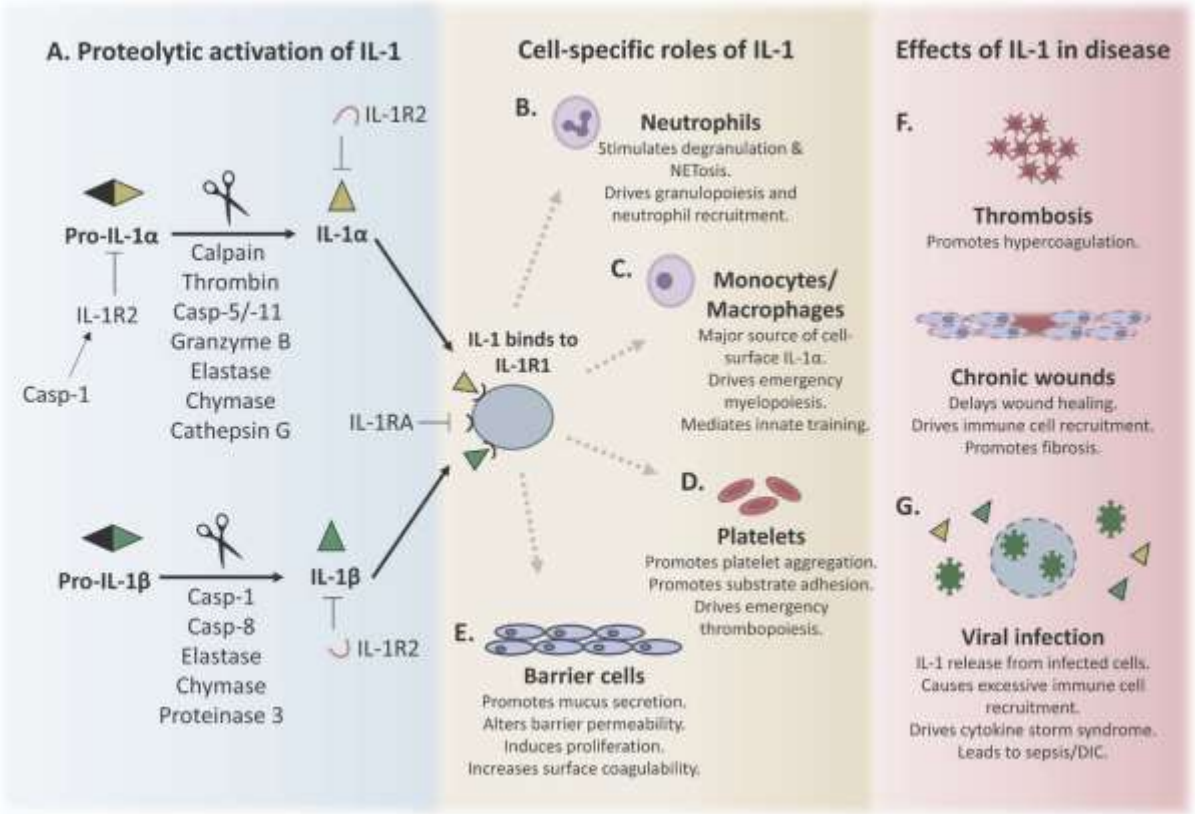

Figure 3. Interleukins-1α (IL-1α) and interleukins-1β (IL-1β).

One of the key functions of interleukins-1α (IL-1α) and interleukins-1β (IL-1β) is to recruit and activate neutrophils or polymorphonuclear leukocytes (PMNs), which are critical for the early stages of the inflammatory response. Upon activation, polymorphonuclear leukocytes (PMNs) migrate to the site of injury, where they phagocytose bacteria and damaged tissue, produce reactive oxygen species (ROS), and release proteases and other enzymes that degrade extracellular matrix components. Interleukin-1β (IL-1β) itself is particularly potent at inducing the migration of neutrophils to sites of inflammation, and it can also increase their lifespan and promote their activation. This activation can result in the release of additional cytokines and chemokines, creating a positive feedback loop that amplifies the inflammatory response. Although Interleukins-1α (IL-1α) can also stimulate neutrophil migration and activation, the effect is to a lesser extent than interleukin-1β (IL-1β).

Studies have shown that in response to scabies infestation, there is an increased presence of neutrophils at the site of infection.[12] An overpopulation of neutrophils can disturb and delay the normal process of wound healing in several ways. Firstly, excessive neutrophils at the wound site lead to increased inflammation, which can damage the surrounding healthy tissue and delay the healing process. Although inflammation is a necessary process during the early stages of wound healing, excessive inflammation can interfere with the subsequent stages of healing. Secondly, neutrophils release reactive oxygen species (ROS) and proteases,[13,14] which are enzymes that can damage the extracellular matrix and surrounding healthy tissue. Reactive oxygen species (ROS) also inhibit the growth and activity of new blood vessels,[15] which are crucial for providing oxygen and nutrients to the healing tissue. Thirdly, neutrophils release cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α), which further promote inflammation and interfere with the normal process of wound healing, as discussed earlier. Fourthly, neutrophils undergo a process called "NETosis" (**see Figure 4**) where the overpopulation of neutrophiles release web-like structures called neutrophil extracellular traps (NET),[16] which indeed trap and kill the scabies mites, but in the process they also cause further damage to the surrounding tissue, lead to the formation of the so-called "DNA/anti-DNA" complexes which stimulate the production of autoantibodies and contribute to further inflammatory response, and certainly delay the healing process. Lastly, prolonged overpopulation of neutrophils lead to delayed clearance of apoptotic neutrophils, which can cause further tissue damage and delay healing.

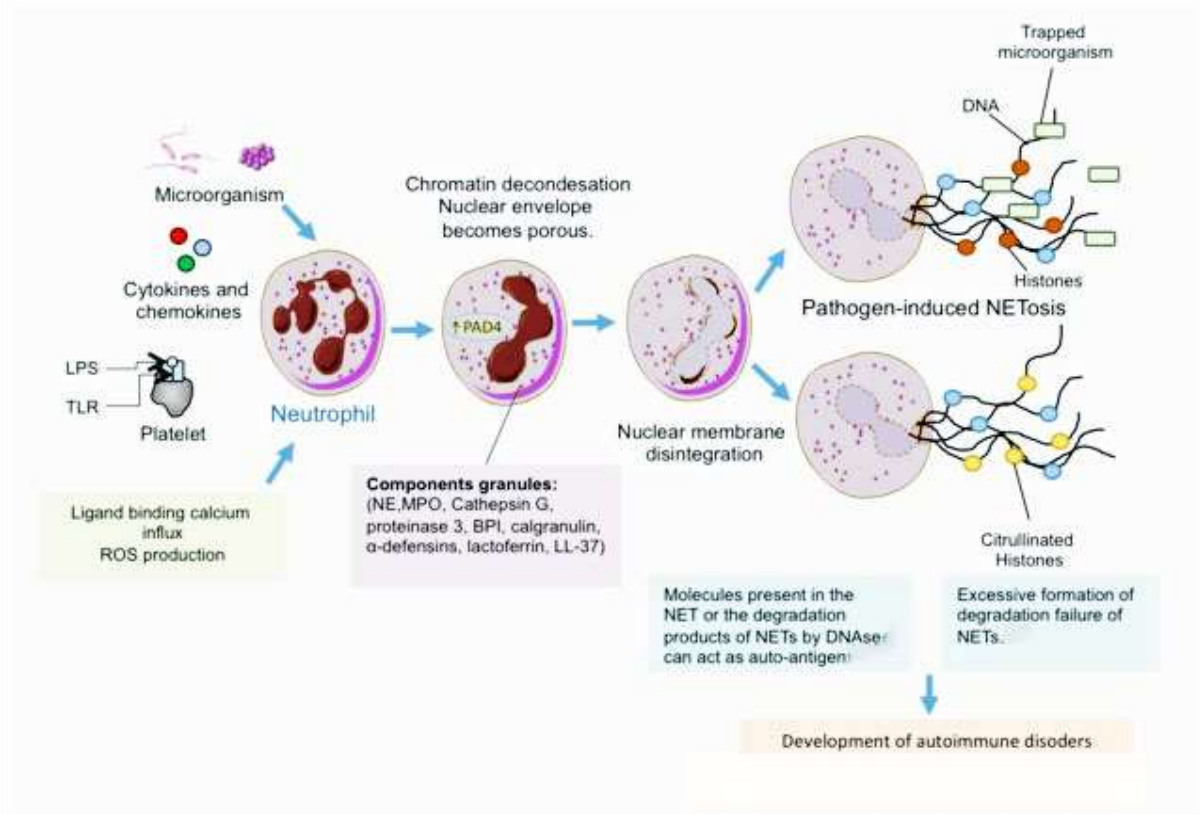

Figure 4. Neutrophile extracellular trap (NET) and NETosis

Effect of scabies comorbidity and proliferation phase of wound healing

In addition to its effects on inflammation, interleukins-1α (IL-1α) and interleukins-1β (IL-1β) can also directly inhibit the migration and proliferation of fibroblasts, which are essential for the production of extracellular matrix components and wound closure. The first mechanism in which interleukins-1α (IL-1α) and interleukins-1β (IL-1 β) inhibit the migration and proliferation of fibroblasts is by recruiting inflammatory cells that produce matrix metalloproteinase (MMP) enzymes,[17] inducing the expression of those enzymes which later degrade extracellular matrix (ECM) components, such as collagen and fibronectin, and providing crucial control for tissue remodelling. However, extreme upregulation of interleukins-1α (IL-1α) and interleukins-1β (IL-1 β) leads to excessive MMP activity and unnecessary degradation of the extracellular matrix (ECM) scaffold that is essential for fibroblast migration and proliferation and therefore results in restriction of the migration and proliferation of fibroblasts.[17,18]

The second mechanism in which interleukins-1α (IL-1α) and interleukins-1β (IL-1 β) inhibit fibroblast migration and proliferation is by inhibiting the expression of growth factors and extracellular matrix proteins. Studies agreed interleukins-1β (IL-1 β) inhibit the expression of both transforming growth factor-beta (TGF-β),[19] a growth factor that stimulates fibroblast migration and proliferation, and fibronectin, an extracellular matrix (ECM) protein that plays a critical role in wound healing. Interleukins-1α (IL-1α) has also been shown to inhibit TGF-β expression and signalling, and to decrease the production of hyaluronan, a component of the extracellular matrix (ECM) that is important for fibroblast migration and proliferation.[20]

Interleukins-1α (IL-1α) and interleukins-1β (IL-1β) can also inhibit fibroblast migration and proliferation by inducing fibroblast apoptosis, or programmed cell death. IL-1β can activate the caspase-3 pathway, which leads to cleavage of cellular proteins and DNA fragmentation. IL-1α has also been shown to induce apoptosis in fibroblasts, although the exact mechanism is not yet fully understood.[21,22]

Oxidative stress

Oxygen (O_2) is a very important compound to produce high levels of adenosine triphosphate (ATP) during the mitochondrial energy-producing sequence, it also plays a role in meeting the high energy requirements for tissue renewal. O_2 has a radical derivative known as reactive oxygen species (ROS), the by-products of mitochondrial oxidative phosphorylation while generating adenosine triphosphate (ATP). Reactive oxygen species (ROS) is the term that applies to molecules in which oxygen (O_2) molecules get reduction with the addition of electrons, which makes these molecules highly reactive and radicals. Examples of reactive oxygen species (ROS) are singlet oxygen ($1O_2$), superoxide anion (O_2^-), hydroxyl ion (OH^-), hydroxyl radical (*OH), hydrogen peroxide (H_2O_2), peroxide (ROOR) and peroxyl radical (ROO*). Reactive oxygen species (ROS) take

electrons from other molecules through an oxidation reaction as the name implies, which destroys their structure.

Human body needs reactive oxygen species, in certain level. A very low level induces physiological cell arrest, a basal level maintains normal cell function and homeostasis, an elevated level leads to cell-mediated immune response and phagocytosis, and a higher level activates pro-apoptotic proteins to induce subsequent cell death. Reactive oxygen species (ROS) are also important in tissue renewal processes as secondary messengers, and in hemodynamic function as a regulator of vasoconstriction and vasodilation. However, high and uncontrolled levels of reactive oxygen species (ROS) can risk damaging lipid, protein, and DNA structures, and later present as chronic, degenerative diseases (such as cancer, metabolic syndrome like type 2 diabetes mellitus, hypertension, Alzheimer disease), and accelerate aging. Leading to early ischaemic episode or reperfusion injury of vital organs such as heart, lungs, kidney, or liver.[23,24,25]

The mechanism of the immune response against scabies mites and to its treatment have other oxidative stress activities. Immune cells especially neutrophils and monocytes have key functions in killing invading microorganisms and regulating the inflammatory response. One of the key mechanisms of the innate immune system is a process called respiratory burst (**see Figure 5**), a rapid release of the reactive oxygen species (ROS) from neutrophils. The capability of neutrophils to destroy bacteria or other pathogens depends on their ability to promote effective respiratory bursts.

As we previously discussed, scabies mite invasion triggers an inflammatory response resulting in increased and sustained production of pro-inflammatory cytokines such as interleukins-1α (IL-1α), interleukins-1β (IL-1β) and tumor necrosis factor α (TNF-α) inducing a response by neutrophils, and macrophages in local lesions. Not only sustained upregulation of interleukins-1α (IL-1α) and interleukins-1β (IL-1β) can lead to delayed wound healing, but also it can prompt chronic inflammation.[6,10,11] Chronic inflammation can result in excessive production of free radicals called reactive oxygen species (ROS) and reactive nitrogen species (RNS) that cause oxidative damage to proteins, lipids, and DNA. This oxidative stress can impair cellular function and further exacerbate the inflammatory response.[26,27] Reactive oxygen species (ROS) originated in cells are called endogenous ROS, while ones from extracellular are exogenous ROS. Most of the endogenous ROS arise as a by-product of mitochondrial oxidative phosphorylation while generating ATP from the endoplasmic reticulum or oxidoreductase enzymes, other known sources are peroxisomes, endoplasmic reticulum, and phagocytic cells (**see Figure 5**). Studies have identified immune cell activity, inflammation, infection, ischemia, cancer, aging, mental stress, and excessive physical exercise can trigger production of endogenous free radicals. The faster metabolism occurs, the more it produces reactive oxygen species (ROS). Meanwhile, the triggers of exogenous free radicals are pollution, tobacco smoking, alcohol, radiation, transition and heavy metals, industrial solvents, pesticides, and drugs such as halothane and paracetamol.[28] Most of anti-scabies topical agents are pesticides which can also trigger exogenous free radicals.

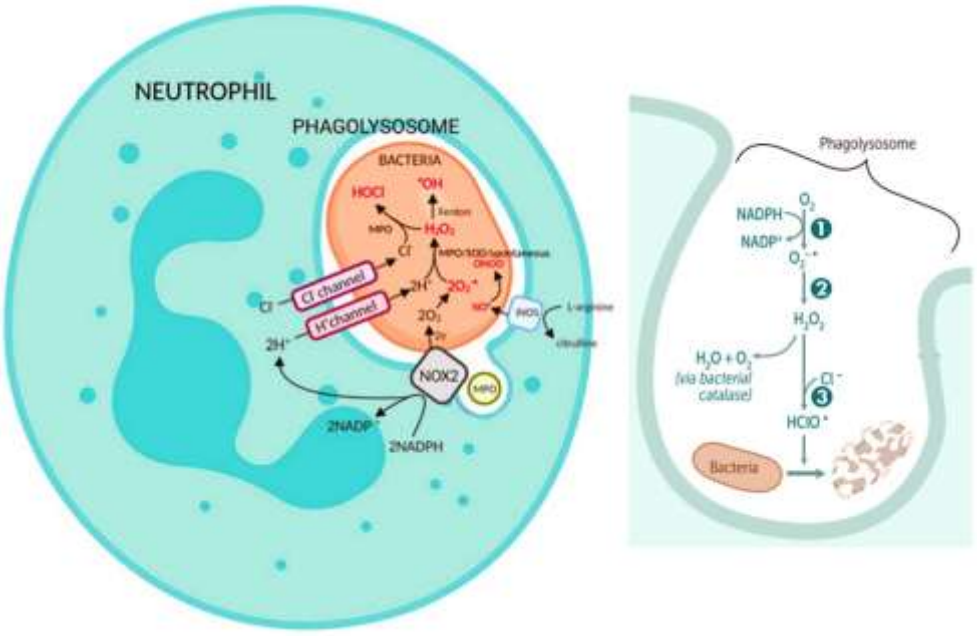

Figure 5. Reactive oxygen species and respiratory burst.

Scabies treatment and oxidative stress

Treatment of scabies is primarily topical using compounds lethal to arthropods and relatively less toxic to humans such as sulfur, benzyl benzoate, lindane, and permethrin. The most utilized topical agents are lindane and permethrin, as other agents have less favourable matters such as repeated

application requirements, low efficacy, the emergence of drug resistance in parasites, and leading tolerability for reduced patient adherence and parasite clearance.

Scientists agreed enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) provide protection from the damaging effects of reactive oxygen species. Anti-scabies pesticides exert deleterious effects to the mites by mechanism of modifying those free radical scavenging systems, although most studies are mostly in experimental animal tissues.[4,29] Topical application of lindane 1% is associated with significant lipid peroxidation, but no significant difference after permethrin 5% exposure. Superoxide dismutase (SOD) and catalase (CAT) activity also increased significantly following lindane 1% application, but no significant difference after permethrin 5% exposure. The increase in the activity of antioxidant superoxide dismutase (SOD) and catalase (CAT) enzymes accompanied by a decrease in glutathione (GSH) levels overcome the excessive free radical load. Although studies showed quite a significant change in the post-treatment radical scavenging system, one interesting fact was the pre-treatment levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity in scabies subjects were not significantly different from controls – meaning scabies per se does not alter the body's antioxidant status.[30]

SUMMARY

Scabies infection elicits a complex immune response involving multiple layers of defense mechanisms. The skin's defense mechanisms are critical in detecting antigen or allergen exposure and initiating an immune cell response. T-cells and keratinocytes contribute to the immune response by producing cytokines. Scabies mite proteins play an essential role in modulating cytokine and chemokine secretion, therefore coexisting scabies infestation can impact wound healing by causing delayed healing and further damage due to not only intense itching and scratching but also due to alteration of inflammatory process responding to scabies mites which trigger upregulation of pro-inflammatory cytokines and reactive oxygen species (ROS), leading to oxidative stress and impairment of cellular function. Anti-scabies topical treatment exert deleterious effects to the mites by modifying the free radical scavenging systems therefore providing protection from the damaging effects of reactive oxygen species (ROS) overproduction. Comprehending the immunological state and oxidative stress in scabies infestation is essential in planning proper management for every wound with scabies comorbidity.

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