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Review article

Why are people with glycative stress so susceptible to COVID-19 infection?

Kyle Haasbroek, Masayuki Yagi, Yoshikazu Yonei

Anti-Aging Medical Research Center/Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Abstract

People with diabetes show an increased susceptibility to infection by SARS-CoV-2, greater incidence of pneumonia, and worse clinical outcomes. As diabetes involves high glycative stress, here we present a review of the literature regarding the potential interactions of glycative stress and COVID-19 that may help to explain some of the observed differences in outcomes of diabetic patients. Glycative stress directly suppresses immune function, leaving the body less able to deal with infection. Increased colonization of potentially pathogenic bacteria, mediated by glycative stress, such as *Staphylococcus aureus*, may also lead to negative outcomes during infection. The presence of *S. aureus* on the skin may weaken its barrier function and increase the risk of infection through the skin. Proteolytic activity necessary for the virus to enter cells may be enhanced in tissue exposed to *S. aureus* as well as by the bacteria's own secreted proteases. Finally, *S. aureus* carriage could be a risk factor for the development of secondary bacterial pneumonia during primary COVID-19 infection. In order to avoid infection and severe disease outcomes, it is important for those suspected of having diabetes to maintain strict glycemic control and take measures to avoid exposure to the virus.

KEY WORDS: COVID-19, SARS-CoV-2, diabetes mellitus, *Staphylococcus aureus*, biofilm, pneumonia, glycative stress, advanced glycation endproducts (AGEs)

Introduction

It is said that people with diabetes, a representative condition with high glycative stress, are more likely to have coronavirus induced (COVID-19) pneumonia, and coronavirus pneumonia is more likely to become severe[1-4]. Although it is a subject that still remains unclear, we would like to present a literature review of this issue.

Aichi Medical University conducted a large-scale survey (45,000 cases or more) on the causes of death in diabetic patients. As a result, malignant tumors were observed in 38% of cases, followed by 17% of cases involving infectious diseases such as pneumonia[5]. It has been reported that people with diabetes are about 1.8 times more likely to have pneumonia than people without it. Infections as complications of diabetes investigated in Japan consisted of pneumonia (respiratory infection): about 40%, urinary tract infection: 25%, skin and soft tissue infection: 20%.

There are many factors involved in cases when it comes to diabetes. The situation is quite different for people who are properly treated and have good glycemic control, those who are being treated but have insufficient glycemic control, and those who are left untreated for hyperglycemia. Naturally, people with severe hyperglycemia have a higher risk of pneumonia and a higher mortality rate[6]. Therefore, diabetics should have strict glycemic control, avoid postprandial hyperglycemia, and carefully and voluntarily take precautions to avoid infection.

Reduced immune function

Why are people with diabetes prone to infections? The human body is constantly attacked by viruses and bacteria that try to enter it, so it has a sophisticated defense mechanism against infection. However, diabetes has weakened the defense mechanism against infection.

Contact Address: Professor Yoshikazu Yonei, MD, PhD
Anti-Aging Medical Research Center/Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tatara Miyakodani, Kyotanabe, Kyoto, 610-0394 Japan
TEL/FAX: +81-774-65-6394 e-mail: yyonei@mail.doshisha.ac.jp
Co-authors: Haasbroek K, cygd2001@mail4.doshisha.ac.jp;
Yagi M, myagi@mail.doshisha.ac.jp

Immune function is the most important defense mechanism. Various types of immune cells cooperate with each other while fighting against external enemies, *i.e.*, bacteria, viruses, and cancer cells. When glycative stress, *i.e.*, diabetes, becomes strong, individual immune cells are affected and immune function declines.

There are two main mechanisms by which the function of immune cells is reduced by glycative stress (***Fig. 1***). The first pathway is mitochondrial function decline. The second is a pathway that causes endoplasmic reticulum (ER) stress in which advanced glycation endproducts (AGEs) produced by glycative stress are taken up into the cell, the burden of foreign matter processing increases, and cell function declines.

First mechanism: Hyperglycemia and AGEs severely damage mitochondria. Inside the mitochondria there is a system of energy production called the TCA cycle. When the TCA cycle is impaired, energy production is reduced and the cells become impaired. In this state, fumaric acid increases and reacts with protein to produce succinylated protein (also called 2SC protein)[7]. When an important protein such as an enzyme is denatured by 2SC modification, the function of that enzyme is impaired. As a result, the functions associated with that enzyme are diminished.

Second mechanism: AGEs are taken into cells through cell surface scavenger receptors[8, 9]. A scavenger is a cleaner, and it is a receptor for processing foreign substances, in this case AGEs. When AGEs increase in cells, the burden of foreign matter processing increases and cell function declines[10]. This condition is called ER stress. The ER is an organelle in the cell called the endoplasmic reticulum, where proteins are processed and synthesized.

Glycative stress impairs the function of immune cells. White blood cells that compose immune cells include neutrophils, macrophages (cells in which monocytes have evolved), lymphocytes, and natural killer (NK) cells. AGEs impede the proper functioning of these cells in various ways.

"Decreased phagocytosis"

When viruses and bacteria enter the body, neutrophils and macrophages take them into cells and degrade them. This is called phagocytosis. If this function declines, the ability to kill pathogens will be weakened. Phagocytic activity is significantly decreased in individuals with diabetes, and AGEs directly suppress phagocytosis in macrophages[11]. Further research demonstrates that incorporation of AGEs into macrophages causes apoptosis[12], reducing the number of viable macrophages during high glycative stress.

"Reduced chemotaxis of neutrophils"

Chemotaxis is the function of moving toward an inflammatory site. Neutrophils are characterized in that they

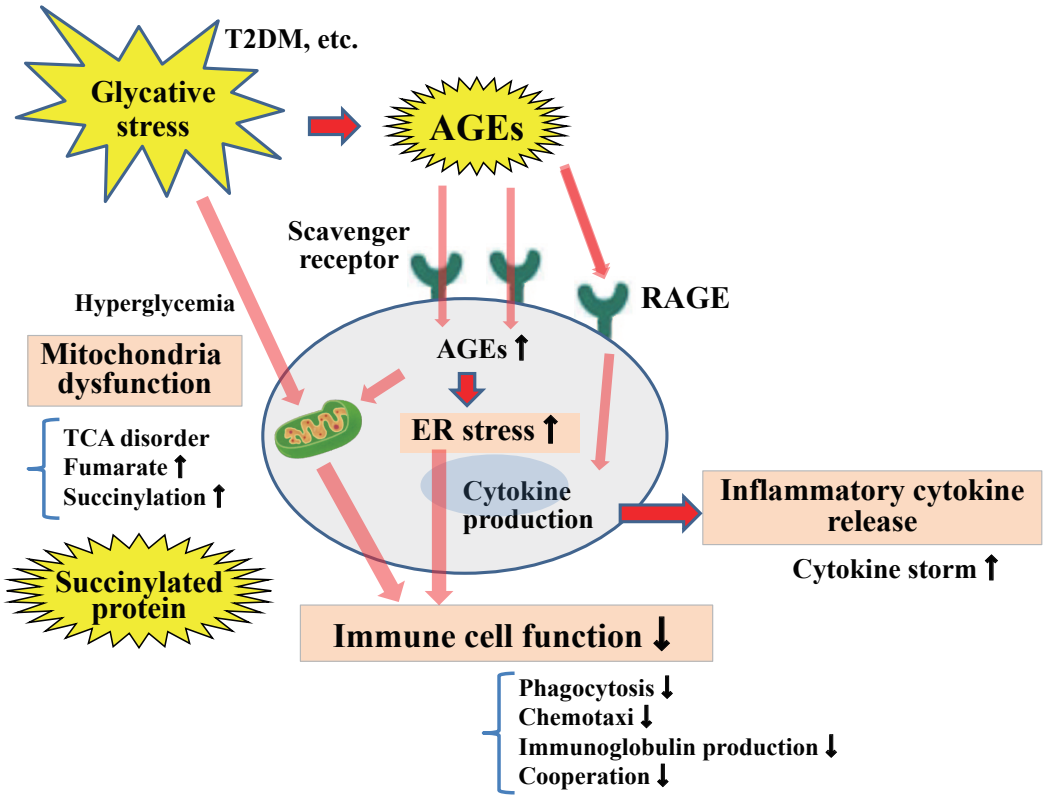

Fig. 1. **Glycative stress and the function of immune cells.**

AGEs, advanced glycation endproducts; RAGE, receptor for AGEs; T2DM, type 2 diabetes mellitus; TCA, tricarboxylic acid; ER, endoplasmic reticulum.

first gather at the site of pathogen entry and inflammation and play a role in the initial response during infection. Neutrophils exposed to diabetic donor serum show reduced migration in response to chemical signals [13], and activation of RAGE (receptor for AGEs) by AGEs increases binding to collagen, reducing chemotaxis in the extracellular matrix [14]. When this function declines, the cell migration speed slows down and the immune response is delayed.

"Lower immunoglobulin production"

Antibodies are proteins, called immunoglobulin, that bind to bacteria and viruses and work to defeat foreign enemies. Antibodies are produced by B lymphocytes. As other factors, neutrophils and NK cells produce free radicals and cytokines, *i.e.* TNF-α, thus exert bactericidal and viricidal action. When glycative stress is high, production and secretion of these substances are reduced [15-18], and the ability to kill pathogens is weakened.

"Reduced cooperation"

Immune cells play their role in cooperation with each other. The cells signal and detect each other, sometimes enhancing bactericidal ability and sometimes controlling inflammation.

However, if glycative stress is strong, the signal response will be impaired and the cooperation will be hindered. As a result, the defensive power against viruses and bacteria and bactericidal ability is reduced, so that infections are more likely to occur.

When AGEs bind to the RAGE, it increases the production of cytokines that cause inflammation. Inflammatory cytokines generally act competitively with insulin, which raises blood sugar levels and further impairs blood sugar control. In addition, inflammatory cytokines may be over secreted. This is called a cytokine storm [19] and is a major factor in the severity of new coronavirus pneumonia.

Effects on skin and mucous membrane defense mechanisms

The transmission route of COVID-19 is mainly droplet infection and contact infection. In the case of droplet infection, patients are infected by inhaling the droplets from a cough and attaching them to the bronchial mucosa, or by attaching the droplets directly to the mucous membranes of their nose or eyes. In the case of contact infection, after contacting the patient or attaching to the skin of the hand through a doorknob or food containing a pathogen, it is transmitted through the eyes, nose or mucous membrane of the oral cavity. Namely, COVID-19 has the characteristic that it easily penetrates through mucous membranes. Among them, the oral mucous membrane is the most susceptible to glycative stress.

When blood sugar level rises, the sugar concentration in saliva rises (saliva hyperglycemia). Therefore, in people with diabetes, periodontal disease bacteria and caries (cavities) may grow, resulting in poor oral hygiene and high prevalence of periodontal disease and caries. These pathogens reproduce by forming a film called biofilm, in which members of the group gather and share nutrient sources with each other.

In this way, they protect themselves from the enemy bacteria and escape the phagocytosis of white blood cells and immunological attacks, *i.e.*, antibodies, cytokines. There are numerous biofilms on plaque, which can cause aspiration pneumonia. The association between biofilms and COVID-19 is unknown at this time, but we suspect that there may be a relationship. Maintaining oral hygiene is important for the prevention of new coronavirus pneumonia.

SARS-CoV-2 Mechanism of Infection

There is a molecular mechanism on the cell surface that allows COVID-19 to enter efficiently. The virus targets the "ACE2 (angiotensin-converting enzyme 2)" receptor as the "entrance" to cells, and utilizes "TMPRSS2" and/or "furin", which are proteolytic enzymes [20]. The viral spike protein binds to ACE2, then TMPRSS2 and furin on the cell membrane cleave the protein at the appropriate position, supporting the fusion of viral and cellular membranes [21]. The virus invades the cell and injects genetic material, RNA. The cell will become a "factory" and will be able to self-replicate large amounts of the virus. SARS-CoV-2 possesses a novel 4 amino acid insert in its spike protein, allowing it to be primed more readily and by a wider variety of host proteases than SARS-CoV-1 [22]. In addition to furin and TMPRSS2, other proteases such as PC1, trypsin, TTSP matriptase, and cathepsins B and L have also demonstrated the ability to activate the viral spike protein.

"ACE2 Expression"

Paradoxically, ACE2 expression is seemingly reduced in long-term diabetes based on murine models [23] (cardiovascular ACE2) and human studies [24] (renal ACE2). However, diabetic patients who are undergoing treatment with ACE inhibitors show the opposite effect: while ACE inhibitors downregulate expression of ACE1, this consequently results in upregulation of ACE2 [25-27]. Despite this potential risk, a study of actual cases involving ACE inhibitor use demonstrated decreased mortality in COVID-19 patients taking the medication [28]. The interaction of the systems of ACE2 expression, diabetes, and COVID-19 are complex and difficult to predict.

Glycative stress also affects the skin's defense mechanism.

COVID-19 is not transmitted through healthy skin. However, if you have diabetes, many people will be aware of symptoms like rough skin, dry skin, and itching. Further disease progress can increase the likelihood of skin infections such as ringworm (athlete's foot) and candidiasis, and sometimes lead to skin erosion. In such areas, the virus can also penetrate through the skin. It is said that infection

is particularly likely to occur on areas of thin skin such as the lips and pubic region. Excessive hand washing, use of alcohol sanitizers, and extended use of PPE (personal protective equipment, *e.g.* masks, gloves) due to the current pandemic are causing an increase in skin damage and dryness, further contributing to skin vulnerability [29].

In our laboratory, we pay attention to the skin-resident microflora and study the changes due to aging and the effects of glycative stress. *Staphylococcus epidermidis and Staphylococcus aureus* reside on the skin in significant quantity. The former is also called "beautiful skin bacteria," a potential probiotic which is common among young people, but decreases with age and diabetes. Conversely, in older people and diabetics, *S. aureus* increases and biofilm formation becomes more noticeable. In our research (currently preparing for publication) we have come to know that AGEs promote biofilm formation. AGEs formed from glucose and keratin cause a roughly 10-fold increase in biofilm formation compared to controls in *S. aureus* at dosages of 0.1~1 mg/mL of keratin. The skin of diabetics, with high AGE content, is ideal for the formation of *S. aureus* biofilms. When combined with skin lesions, an abundance of *S. aureus* and its biofilms may further break down vulnerable skin, slow wound healing, damage the skin's barrier function, and cause inflammation. In addition to the skin, *S. aureus* also inhabits the nasal cavity, which is the primary reservoir for the bacteria on the body of persistent carriers.

The skin itself is rich in RNA and DNA degrading enzymes [30, 31], which may degrade viral RNA and reduce its survival time on the skin surface. Bacterial biofilms are partially composed of extracellular DNA which forms a substantial part of the biofilm matrix [32], and are broken down by ribonucleases [33-35]; these enzymes will be diminished in the environment of a stable biofilm, which may also protect SARS-CoV-2 from degradation on bare skin, allowing it to persist for longer on the skin surface.

Considering these factors, we reviewed the literature for other potential mechanisms that could exacerbate COVID-19 in the case of *S. aureus* carriage. We found that the presence of *S. aureus* and its proteases could play a potential role in increasing susceptibility to SARS-CoV-2. There are two main pathways for *S. aureus* to promote susceptibility to COVID-19: modulating host protease activity, and by priming the virus with its own bacterial proteases (***Fig. 2***).

Exposure to *S. aureus* factors triggers an increase in serine protease activity in human keratinocytes. Trypsin and the KLK family of proteases expression in human keratinocytes was shown to increase *in vitro*, and *in vivo* murine models also showed increased serine protease activity in the skin after exposure to *S. aureus* [36]. Another study of *S. aureus* in atopic dermatitis skin lesions revealed that *S. aureus* strains colonizing lesions demonstrated increased proteolytic activity when compared to reference strains [37]. Tissues experiencing this effect would produce more host protease, increasing viral fusion and infection rate. While there does not appear to be any data on the host protease

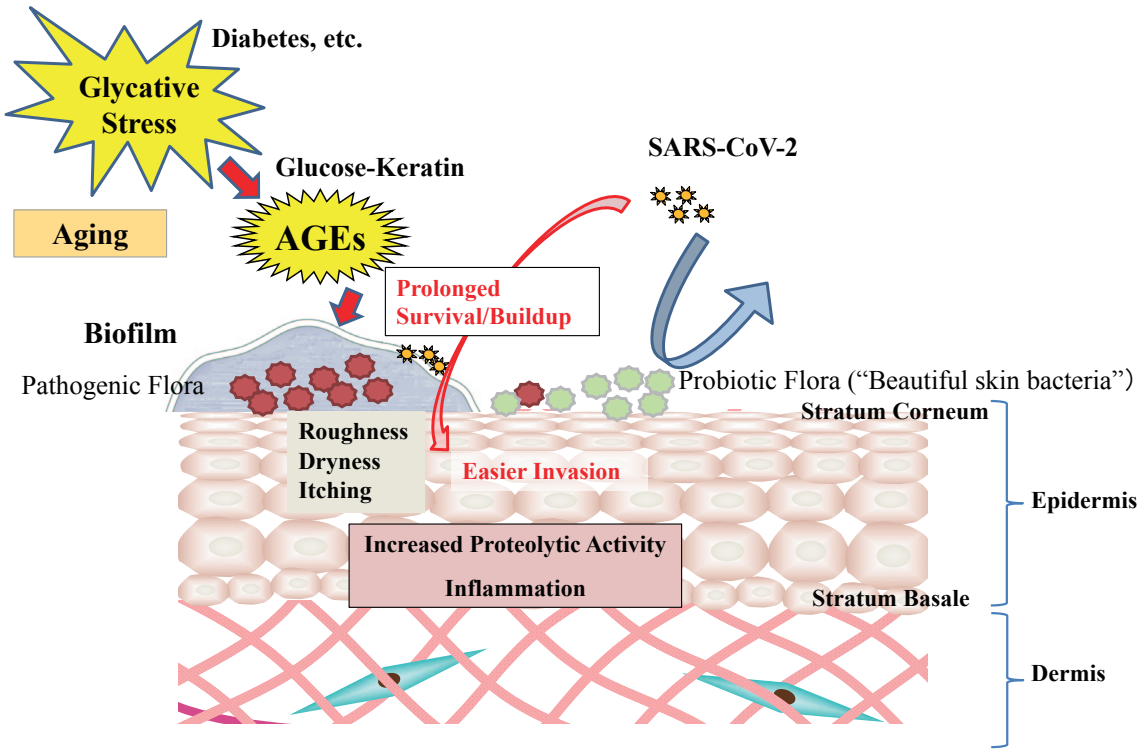

Fig. 2. **Glycative stress and the defense mechanism in skin.**

AGEs, advanced glycation endproducts.

response in the nasal mucosa, the presence of *S. aureus* and its secreted serine proteases in the nose have been shown to have an immunomodulatory effect on nasal epithelia cells, increasing cytokine production and causing inflammation [38].

Although it seems that there are not yet any studies examining the capacity for bacterial proteases to interact with the SARS-CoV-2 viral spike protein, *S. aureus* relies on a suite of secreted proteases for its nutrient metabolism as well as protection against host defenses [39]. These include two cysteine proteases (staphopain A and B), a metalloprotease (auerolysin), a serine protease (V8), and six serine protease-like proteins (SplA – SplF). It is possible that some of these may have overlapping specificity with the human proteases previously noted to interact with SARS-CoV-2. Additionally, S. aureus also secretes proteins that specifically inhibit neutrophil proteases [40], impeding the ability of the immune system to clear invading pathogens.

Here we have collected recent findings on COVID-19 and skin and mucosal membranes.

S. aureus secondary infection during COVID-19

S. aureus carriage is already a potential risk factor in hospital settings under normal circumstances, being associated with increased incidence of surgery site infections [41, 42], and nosocomial spread of MRSA is a relatively common occurrence. Presence of *S. aureus* is also a risk factor for co-infection with other viral infections; the *S. aureus* protein lipase 1 has been found to increase replication of the Influenza A virus [43], and Influenza can also trigger virulence in commensal nasal *S. aureus* [44], leading to secondary pulmonary infection and bacterial pneumonia. Co-infection is also a serious concern for COVID-19, and similar processes may be a generalizable factor. A thorough meta analysis of COVID-19 patients [45] reveals that 7% of hospitalized patients (and 14% of ICU patients) had confirmed cases of bacterial co-infection, and were at significantly higher risk of death. In some cases, up to 50% of non-survivors of COVID-19 have presented with co-infection [46]. Elevated body temperatures from fever promote the dissolution of *S. aureus* biofilms [44]: if *S. aureus* biofilms are present in the nasal cavity, high concentrations of *S. aureus* cells may be aspirated into the lungs, leading to secondary infection. A fatal case of secondary lung infection by *S. aureus* during COVID-19 has been reported [47].

COVID-19 skin infection

Some hospital dermatology departments have issued reports regarding their concerns of increased vulnerability for their patients who could more easily contract the disease during hospital visits [29, 48]. COVID-19 infection of the skin is theoretically possible. ACE2, while highly expressed in the lungs and nasal mucosa, is expressed throughout many tissues in the body, including the skin [49, 50]. Increasingly there are also reports of skin rashes as an early COVID-19 symptom. A survey through a monitoring application (n = 336,847) reports 8.8% incidence of skin rash in positive cases, and a separate survey of COVID-19 patients (n = 11,546) found that 17% of patients experienced a rash as their first symptom, and 21% as their only symptom [51]. While the pathogenic mechanism of theses rashes/lesions is still unclear, it is hypothesized that they may occur due to a hyperactive immune response and/or vascular damage [52]: not necessarily due to infection of the skin itself. Other case studies have noted the formation of vesicular rashes in some patients [53-55], which form from viral replication in the skin in other disease systems (e.g., *Herpes simplex*, poxviruses, *Varicella zoster*). However, researchers were unable to detect viral RNA in the fluid of these vesicles [53] (although they note the apparent unreliability of skin PCR and question whether low viral load prevented detection of the virus). Nevertheless, infection through the skin is a plausible concern, in addition to the more vulnerable mucosal membranes.

While the risk of infection through skin lesions is unclear, it is clear that mucous membranes are a likely entry point for the virus during infection, especially the nasal epithelia where ACE2 is highly expressed [56]. Both in the nose and on the skin *S. aureus* proteases have the opportunity to contribute to the virulence of SARS-CoV-2. Outside of the nose, *S. aureus* is most highly abundant on the hands, chest, and skin near sebaceous glands and mucous membranes. In either case, *S. aureus* may interact with host tissues and the virus in ways that could increase the susceptibility to and severity of infection.

Reduced blood flow and microcirculatory disturbances

Hyperglycemia causes damage to peripheral nerves. Nerves that feel pain and itching are also damaged, so it may be difficult for symptoms to appear and furthermore becoming difficult to notice skin lesions. These unnoticed skin lesions also provide vulnerable targets for *S. aureus* colonization and infection.

Also, in hyperglycemia, the blood flow through the thin blood vessels of the skin is impaired. In such a state, oxygen and nutrients are not sufficiently distributed, immune cells are less likely to collect at the infected site, and the function of immune cells is reduced. Therefore, pathogens are more likely cause infection.

States with high glycative stress, *i.e.*, obesity [57], glucose intolerance, diabetes [58], not only increase the risk of coronavirus infection, but also increases the frequency of thrombus formation. Thrombus formation becomes a factor of disease severity and increases the mortality rate [59-61]. An important phenomenon in the process in which glycative stress influences thrombus formation is vascular endothelial injury. We have pointed out the possibility that postprandial hyperglycemia (glucose spikes) increases linear glucose and fructose containing exposed aldehyde groups which gradually react with saccharides in blood vessels and sugar chains on cell membranes to simultaneously produce multiple aldehydes (aldehyde spark) [62]. Aldehydes have high reactivity not only with sugar chains but also with proteins, thus inducing extensive damage in the vascular endothelium, where rolling

and sticking of leukocytes appear in advance at the lesion site, and eventually a series of microcirculatory disorders leads to leukocyte adherence, emigration in venules, rouleaux formation of red blood cells, and finally causing platelet aggregation and thrombus formation. We have been studying the microcirculation using *in vivo* microscopy and observed that similar phenomena in the gastric mucosal are caused by administration of ethanol or indomethacin [63, 64]. It is important to prevent endothelial damage due to glycative stress as much as possible in order to prevent thrombosis, a serious complication of COVID-19 infection.

Conclusions

Again, the SARS-CoV-2 virus first attaches to the skin of the fingers, and then enters the body via the bulbar conjunctiva, the mucous membranes of the oral cavity and bronchi, by rubbing the eyes and touching the lips. Whether or not an infection is then triggered depends on whether the immune system can eliminate the virus. When glycative stress is strong, the defense mechanisms of the skin and mucous membranes are weakened and immune function is impaired, so new coronavirus pneumonia more easily develops.

Colonization of the body by poteintially pathogenic flora such as *S. aureus*, increasing with glycative stress, may be a risk factor for co-infection. The presence of bacterial biofilms may reduce skin barrier function and modulate proteolytic activity such that viral infection is more liekly to occur.

People with diabetes (including suspicion of having diabetes) should have strict glycemic control, and people without diabetes should reduce postprandial hyperglycemia.

Conflict of Interest Statement

The authors claim no conflict of interest in this study.

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