ACE-2 receptor expression on the back of the hand: Implications in the indirect transmission of SARS-CoV-2

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Research Article

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Abstract

The SARS-CoV-2, is the causative agent of COVID-19, a disease that was declared a pandemic by the WHO, at the beginning of 2020. Various authors have pointed out that morbidity and mortality, as well as the economic consequences of a pandemic, are influenced by factors such as the susceptibility of the population to infection, the severity of the disease, transmissibility and routes of transmission. It was of particular interest in this work to focus on the last two aspects, and, more specifically, on indirect transmission through contact with the back of the hand, highlighting the importance of this site during the transmission process of SARS-CoV- 2. The expression, as well as the functionality of the ACE-2 receptors on the back of the hand was demonstrated by immunohistochemical techniques, and the implications of these findings in the indirect transmission of the virus were discussed. We conclude that contact with the skin, for example, through the fist salute, contributes to the increase in infections, in such a way that we suggest that this practice be avoided as a protection measure.

INTRODUCTION

The SARS-CoV-2 and its interaction with the receptor ACE-2

The coronavirus that causes severe acute respiratory syndrome, type 2 (SARS-CoV-2), is the causative agent of COVID-19. This virus belongs to the Coronaviridae family(1), which includes the SARS-CoV and the virus that causes Middle East respiratory syndrome (MERS-CoV), al lof them, causing severe clinical syndromes (2). SARS-CoV-2 has a high transmission rate, mainly by aerosols and close contact, and the respiratory tract has been recognized as the main route of entry into the body, through infected people or through contact with contaminated surfaces, where the pathogen remains active for hours. Furthermore, it is well recognized that infected people have mild to moderate respiratory symptoms, after an incubation period of up to 14 days, others face severe symptoms that ultimately lead to acute respiratory distress syndrome (ARDS), associated with a cytokine storm syndrome (CSS)(3).

The SARS-CoV-2 receptor, Angiotensin-Converting Enzyme 2 (ACE-2), which is found in the human respiratory system and with higher expression in older adults than in younger adults, is known to(4), is the anchor point and pathogenicity of this virus. In vitro studies support the interaction between ACE-2 and the protein “*Spike* (S)” or SARS-CoV-2 spike. The surface glycoprotein S of coronaviruses, which mediates attachment to and entry into target cells, is composed of two subunits, S1 and S2. The S1 subunit contains an N-terminal domain (NTD) and a receptor-binding domain (RBD) encompassing the receptor-binding motif (RBM). The S2 subunit contains a fusion peptide (FP), hepta-repeat domains 1 (HR1) and 2 (HR2), and a transmembrane (TM) and cytoplasmic (CP) domain (5, 6).

After S1 binds to a membrane receptor, FP inserts into the cell membrane to promote fusion with the viral membrane, a process that depends on proteolytic cleavages at the S1/S2 site to separate S1 and S2 and into the S2 site to generate a mature FP (7, 8).

Although the ACE-2 receptor is typical of type II pneumocytes and bronchial hair cells, it is not exclusive to the respiratory system; since there are other tissues and organs that express said receptor, such as the heart, kidneys, liver, intestines, oral cavity, nasal, brain, thyroid, stomach, reproductive systems, lungs, pancreas, eyes and skin (9) (Fig. 1).

Figure 1.

ACE-2 expression in the skin.

Regarding the expression of ACE-2 in the skin, it has been shown that it is present in the basal cell layer of the epidermis, extending to the basal cell layer of hair follicles. Smooth muscle cells surrounding sebaceous glands are also positive for this receptor. In addition, a strong granular staining pattern for ACE-2 has been seen in Organs that express ACE-2 in cells of the eccrine glands (10), however, little is known about the distribution of SARS-CoV-2 in different regions of the skin.

In the literature it has been reported that the skin is not uniform, it presents differences in its permeability, activity of the immune system and even its microbial composition. (11). The distribution of sebaceous cells seems to be an important point, since areas rich in sebaceous glands are characterized by weaker permeability barrier characteristics, mainly due to lower expression of tight junction proteins (11). It is for this reason that it is important to analyze specific epithelial regions, which may be more susceptible to SARS-CoV-2 infection or, failing that, be a site of probable transmission; such is the case of the back of the hand. In our work group, we analyzed the expression of ACE-2 in a group of tissue biopsies from the back of the hand, in order to discuss the importance of said tissue during the transmission process of SARS-CoV-2.

ACE-2 expression on the back of the hand.

Samples from 5 patients with a presumptive diagnosis of actinic keratosis who attended the Hospital Regional de Alta Especialidad (HRAEI), undergoing skin excision biopsy procedures for diagnostic corroboration purposes. From the samples obtained, areas of healthy skin were selected, which were used to perform immunohistochemistry. All procedures and use of tissue (anonymized) were performed in accordance with recent national ethical guidelines. The mean age of the patients was 40 to 55 years and the male:female ratio was 3:2. All the individuals included in the study signed the informed consent, with the endorsement of the Research Ethics Committee of the HRAEI.

ACE-2 receptors were shown to be present on epithelial and endothelial cells in samples taken from the back of the hand of the patients included in the study. Cells and membranes were stained, denoting the presence of ACE-2; positive staining for ACE-2 was observed in myofibroblasts and in the endothelial cell membrane, being distributed throughout the observed skin area. Cytoplasm of epithelial cells on the back of the hand also showed weak positive staining for ACE-2. In skin, ACE-2 was present in the basal cell layer of the epidermis extending into the basal cell layer of hair follicles. (Figs. 2A, 2B and 2C). Smooth muscle cells surrounding the sebaceous glands were also positive for ACE-2. Weak cytoplasmic staining was lost in sebaceous gland cells, as well as a strong granular staining pattern for ACE-2 in eccrine gland cells. (Fig. 2B).

Marked ACE-2 immunostaining was found in epidermis, epidermal keratinocytes, melanocytes, and basal cells. (Figs. 2A **y 2B**).

This indicates that, in the epithelial tissue of the back of the hand, the ACE-2 receptor is expressed, turning this area into a site where SARS-CoV-2 could adhere.

Figure 2.

Spike protein binding to ACE-2, on the back of the hand.

To reinforce the idea that the back of the hand may be a potential adhesion site for SARS-CoV-2, we decided to test the ability of the SARS-CoV-2 Spike protein to bind to the ACE-2 receptors on the back of the hand. hand in hand.

For this we immunostained the biopsies, this time using the recombinant protein Spike-RBD from SARS-CoV2, which contained a histidine tag. Subsequently, we incubated with anti-His Tag Antibody. Positive immunoreactivity was manifested by brown staining.

The results were positive for the Spike-RBD marker binding to the ACE-2 receptors present on the back of the hand, previously identified in this study. In the photographs obtained (Fig. 3) the stratified layer of epidermis is clearly observed, in its subset with the lucid layer and part of the granular layer, as well as the spinous layer, observing granular keratinocytes, Langerhans cells and spiny keratinocytes with positive brown staining; some cells of the basal stratum are also stained, as well as some melanocytes. Intensive staining can be identified on images.

Figure 3.

Skin implications during the transmission process of SARS-CoV-2.

SARS-CoV-2, like other respiratory viruses, such as influenza and rhinoviruses, can be transmitted independently and simultaneously by several routes, either directly or indirectly. Direct transmission occurs through exhaled droplets or aerosols that reach the respiratory tract or through physical contact between a susceptible person and an infected person.; indirect transmission occurs through contact with contaminated surfaces or objects and subsequent autoinoculation into mucous membranes or by serial transfer via fomites (12–14).

Although the main route of transmission is through the air, it has been suggested that indirect contact, through surfaces, including the surface of the skin, contributes considerably to the transmission of the virus. (15).Such is the potential impact of indirect transmission that, among the most affordable non-pharmacological intervention practices to prevent and control the transmission of SARS-CoV-2, is the disinfection of inanimate surfaces and hands (15).

In this regard, since SARS-CoV-2 is remarkably stable on human skin, hand disinfection reduces the risk of indirect transmisión (14, 16). This is particularly important in a country like Mexico, where the fist salute was established after the arrival of the Omicron variant, despite the fact that it has been suggested that the skin is a potential host for SARS-CoV. -2(Sun et al., 2020).

In fact, the virus has been shown to be highly stable on the skin, suggesting its potential role in dermal transmission and the high rate of spread of the virus (16). The suggested transmission mechanism involves the sneezing or coughing of an infected individual, which can contaminate a surface or directly the skin of another individual. The virion can remain intact and infectious on skin (either sebaceous, oily, or clean skin with exposed stratum corneum), so the individual can become infected by touching their eyes, mouth, or nose (12).

DISCUSSION

Although there is no concrete evidence of the tropism of SARS-CoV-2 in the skin, it is known that this is determined by the tissue distribution of ACE-2. (18).The studies carried out in our laboratory confirmed the expression of ACE-2 on the back of the hand and its functionality after binding to the Spike protein (Figs. 2 and 3), opening the possibility of the presence of SARS-CoV-2 at this site and its potential transmissibility.

Transmissibility depends on the characteristics of the virus, including its infectivity and the environmental stress exerted on it during transmission, as well as factors attributable to the population, including the contagiousness of the infected individual, the susceptibility of the exposed individual, and contact patterns between infected and exposed individuals (13).

Regarding viral characteristics, some studies have shown the stability of SARS-CoV-2 on skin. A study, carried out with a skin model, which replicates in vivo hand skin conditions, revealed that SARS-CoV-2 has a survival of 9.04 hours (IC 95% 7.96–10.2) and a half-life of 3.53 hours (IC 95% 3.02–4.16) suggesting its potential role in skin transmission and in the high rate of spread of the virus (16). Another study reported that the virus persisted for 96 to 168 hours on the skin of pigs, with a half-life of 3.5 hours at room temperature, and even longer at low temperatures. (46.8 hours a 4°C) (19).Taking into account that SARS-CoV-2 is present in the skin, it has been hypothesized that, if this organ is involved in the transmission of the virus, the skin barrier function could be the mediator(20).

In relation to contact patterns, although it is worth noting the contribution of casual contact with contaminated surfaces, there are several limitations to its study. On one side, the frequency and manner of contact with these surfaces is variable and depends, to a large extent, on age, personal habits, type of activities, personal mobility and the level of cleanliness of the environment. On the other hand, it is difficult to distinguish between different routes of transmission, such as person-to-person transmission or autoinoculation (21).

However, as pointed out Boone & Gerba (2007)(21), various investigations support indirect transmission, under the following statements: (I) most respiratory viruses can survive on fomites and hands for variable periods of time; (II) fomites and hands can becoming contaminated with viruses from both natural and laboratory sources; (III) viral transfer from fomites to hands possible; (IV) the hands come into contact with the portals of entry of the viral infection; and (V) disinfection of fomites and hands interrupts viral transmission.

CONCLUSIONS

In Mexico, after the third wave of COVID-19, the fist salute began to be used, which involves contact of the back of the hand, between two individuals. With this, it was intended to avoid the spread of SARS-CoV-2, however, our research suggests that this greeting may be a source of transmission of this pathogen. Coupled with poor handwashing practices and the inappropriate use of disinfectants, such as alcohol-gel, indirect transmission through skin contact with surfaces and subsequent autoinoculation, for example, by rubbing the eyes or putting fingers to the nostrils, favor the roles of contagion.

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Figures

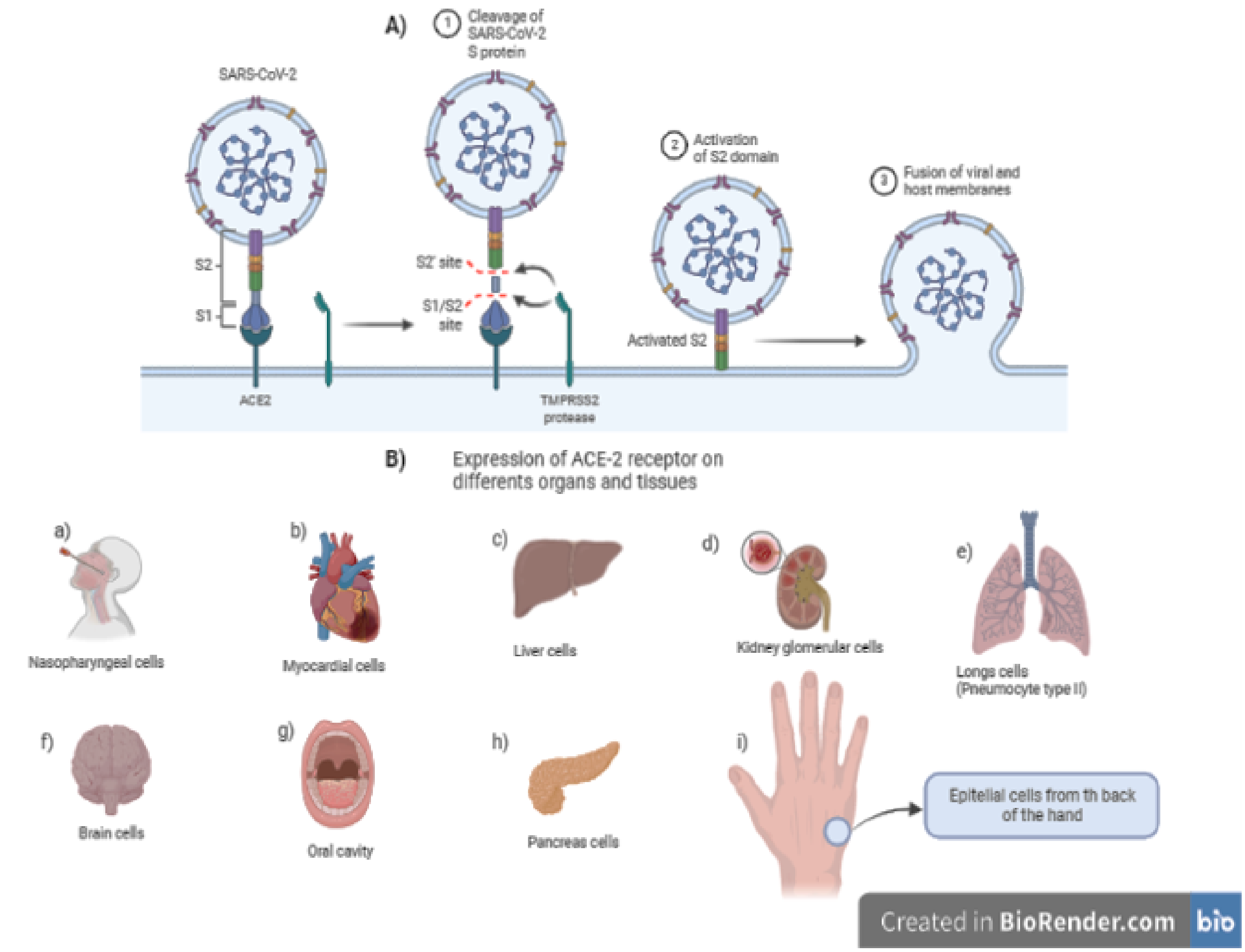

Figure 1

A)Union and entry of SARS-CoV 2 to type II pneumocytes via the ACE2 receptor.

B) Organs that express ACE2

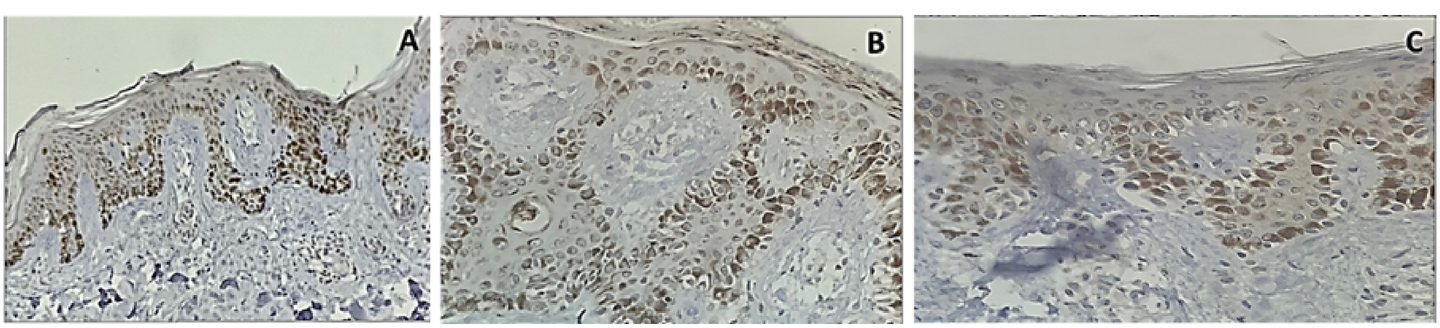

Figure 2

Immunohistochemical expression of ACE2 in healthy skin samples from the back of the hand, obtained by biopsy. **A)** Panoramic vision (4x). ACE2 receptor expression was demonstrated; a pattern of positive staining both cytoplasmic and nuclear is observed. **B).**Microscopic Vision (40x). The greatest positivity is detected in the different cells of the epidermis: epidermal keratinocytes, melanocytes and basal cells. **C)** Microscopic Vision (40x). The maximum expression of immunostaining of the ACE2 receptors is observed in the basal cells.

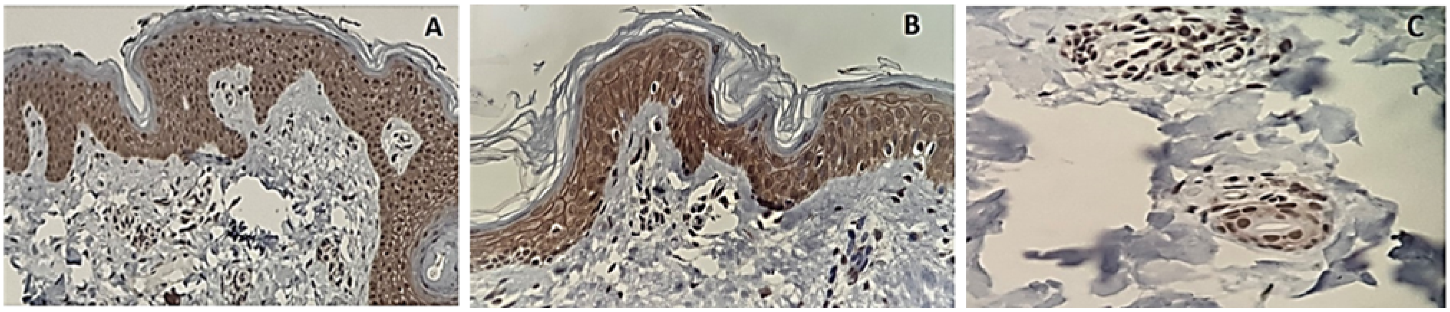

Figure 3

Functionality of the ACE2 receptor, after binding to the Spike protein in samples from the back of the hand. **A)** Microscopic Vision (10x). Positive Observation of cells of the dorsum before the union Spike vs ACE2. **B)** Microscopic Vision (40x). Positive Binding Spike vs ACE2; the highest positivity is detected in different cells of the epidermis, mainly melanocytes. **C)** Microscopic Vision (40x). Internal control with positive staining; endothelial cells lining capillaries, deep superficial dermis.