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Histological Changes in Normal Human Skin: A Biological Marker in the Pathogenesis of Mpox and its Significance in Emerging Health Events

Cambios Histológicos en la Piel Humana Normal: Un Marcador Biológico en la Patogénesis de Mpox y su Importancia en Eventos de Salud Emergentes

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SUMMARY: The body's largest organ, the skin, is also an efficient protective barrier. It consists of the epidermis, dermis, and hypodermis. However, it is also a gateway for infections that cause localized degeneration in its components. Knowledge of standard histological patterns is relevant for understanding the changes observed due to the action of emerging infectious agents, such as monkeypox (Mpox), (WHO, 2024). This study aimed to compare the histological changes between normal skin and those reported in the literature in skin infected with Mpox, thus designing better preventive policies for life and public health. Biopsies of normal human skin obtained from teaching material at the Universidad de Chile were subjected to routine histological techniques. The results show a typical epidermal structure with stratified squamous epithelium, a dermis consisting of dense irregular connective tissue and keratinocytes, and a hypodermis with abundant adipose tissue and blood vessels, with tactile corpuscles observed in the dermal papillae. Nevertheless, in infectious pathologies, the best protection method is to pay attention to personal hygiene, avoid touching used objects, and avoid animal waste.

KEY WORDS: Emerging Pathologies; Public Health; Skin Histology; Biological Markers.

INTRODUCTION

The skin is considered one of the largest organs in the body. From birth to adulthood, it serves as a vital and efficient barrier between the environment and the body, helping to regulate water balance. In its histological organization, from the free surface to the basal layer, there is the epidermis, which corresponds to a stratified, flat, cornified epithelial layer, followed by the dermis, which corresponds to a dense, irregular, collagenous connective tissue with blood vessels, lymphatics, and nerves, while deeper down is the hypodermis (Visscher *et al*., 2015). The embryonic layers of the ectoderm and mesoderm contribute to its formation as it migrates to cells from the neural crest. Molecular alterations play an important role in the skin's biology and biochemistry and the environment's composition (Taïeb, 2018). It is important to consider that the thickness of our epidermis is between 30 and 100 microns, which is significant for drug research, vaccines, clinical research related to the skin and skin transfer operations used in plastic surgery (Oltulu *et al*., 2018).

The ectodermal skin is defined very early during embryonic development, first by the non-neural ectoderm and then by the somite's differentiation in the dermatome's formation that will give rise to the dermis. As embryo-fetal development progresses, hair follicles, merocrine (eccrine) sweat glands, apocrine glands, and sebaceous glands form. In adults, the skin comprises a stratified, cornified, flat epithelial lining, or epidermis, and deeper down, a dense, irregular connective tissue, or dermis. The dermis also contains hair erector muscles, blood vessels, and nerve branches (nerve corpuscles).

The stratified cornified epithelium's epidermis comprises several layers of cells or keratinocytes. The basal

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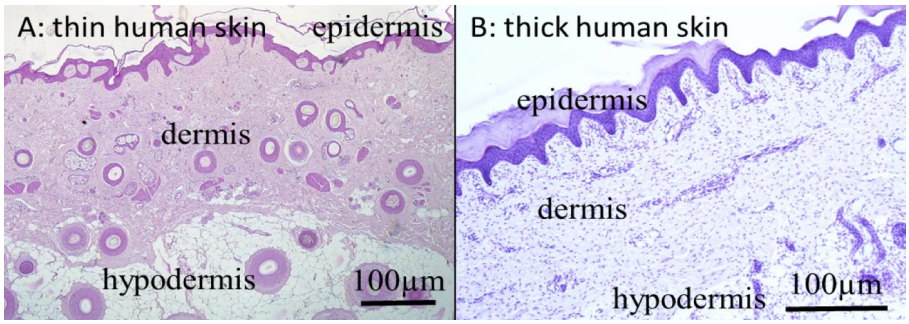

Fig. 1. Human skin biopsy. 1A. Thin skin biopsy. 1B thick skin biopsy. H&E staining.

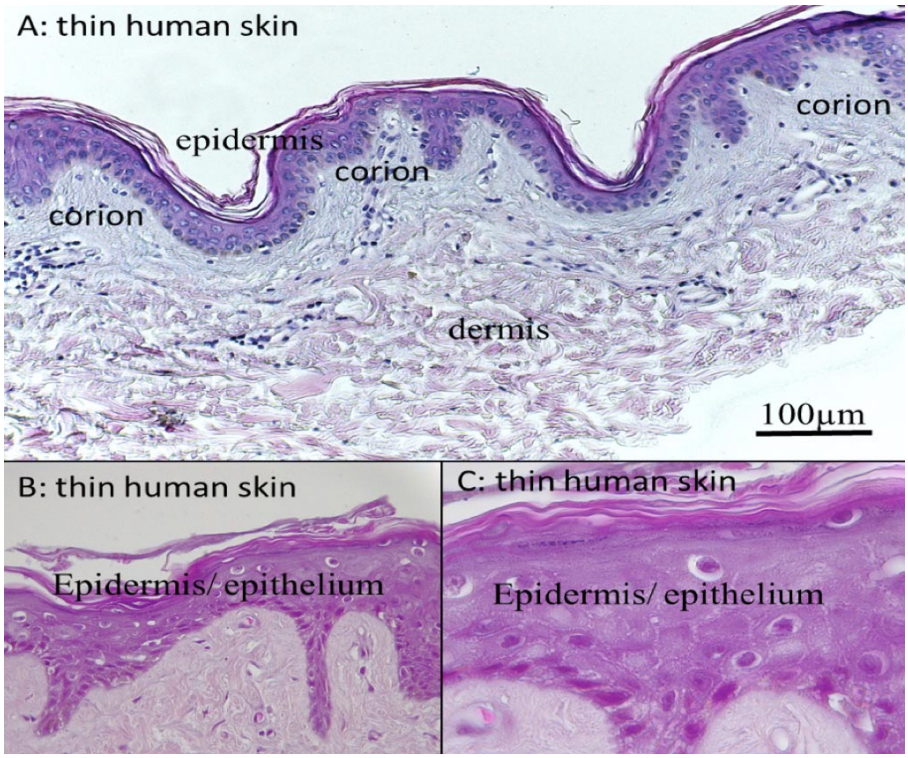

Fig. 2. Biopsies (A, B, C) showing in increasing magnification (40x, 100x) the organization of the epidermis as a stratified squamous epithelium and its relationship with the connective tissue of the dermal papilla or chorion. H&E stain.

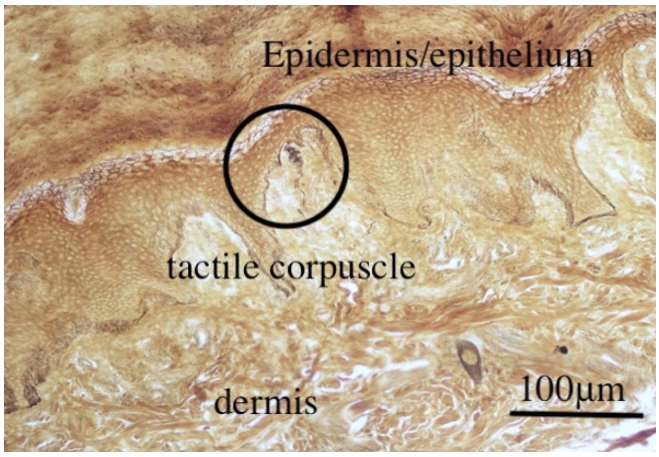

Fig. 3. Human skin biopsy. Epidermis and dermis. Observation of tactile corpuscle (circle). Silver staining.

layer cells are cubic in shape with a rounded nucleus. These cells are the only ones that adhere to the basal lamina through hemidesmosomes (intracellular component) and the type IV collagen basal lamina (extracellular component). This epithelial layer has the highest mitotic index of the epidermis (Figs. 1, 2, 3).

Intrusion by infectious agents into the skin generally occurs through the bloodstream, with previous infections entering through the airways and digestive tract, although contact is also possible. Generally, when these agents come into contact with the skin, they reach the dense irregular connective tissue of the dermal papilla or papillary layer and travel to the basal layer of type IV collagen, passing through it and lodging in the lucid layer, setting up a reactive inflammatory process with an initial accumulation of transudate and subsequent exudate, causing localized degeneration of the skin and mucous membranes. Apoptosis, degradation of the basement membrane, and progressive accumulation of fluid cause the epidermis to lift, revealing the presence of a blister.

Knowing the normal histological patterns of human skin is very important, especially for understanding the changes observed due to the action of various infectious agents, such as monkeypox virus (Mpox), a viral zoonotic disease that has spread rapidly around the world and has been declared an international health emergency by the OMS (OMS, 2022). The main transmission of Mpox in humans is through direct contact with skin lesions, bodily fluids, and contaminated objects, such as

bedding. Percutaneous transmission is less common but can occur when skin barriers are broken, with the risk increasing in situations where medical procedures involve handling patients with infected lesions. Transmission in the usual settings among unvaccinated contacts is 9 % to 12 % (Petersen *et al*., 2019; ECDPC, 2022; Mitjà *et al*., 2023; Kim *et al*., 2023).

Timely diagnosis of emerging infectious diseases is, therefore, essential. The PCR test may be the most suitable tool; however, if this technology is not available, diagnosis can be made through a histopathological study, identifying the specific cytoplasmic changes in these diseases (Alakunle *et al*., 2024; Maronese *et al*., 2022). This study aims to help recognize and detect early changes in skin histology and improve the treatment and management of patients with infectious diseases such as Mpox, thereby designing preventive measures for life and public health.

MATERIAL AND METHOD

Biopsies of adult human skin were obtained from the Teaching Histological Material of the Microteca of the Anatomy and Developmental Biology Program of the Faculty of Medicine, Universidad de Chile. Sections 5 µm thick were impregnated in paraffin with a melting point between 56 and 58 °C. Different slides were subjected to different histological staining techniques to demonstrate general morphology and highlight components of some tissues: hematoxylin and eosin (H&E) staining and toluidine blue staining to describe general morphology, silver staining to observe the lining epithelium and dermal papillae (tactile corpuscles).

Subsequently, observations were conducted using an OLYMPUS CX31 optical microscope with different objectives. The microscope has a built-in AmScope MU1803 digital camera connected via USB 3.0 to a computer (PC) with the AmScope 3.7 for digital camera application.

At the same time, an IN SILICO study was carried out using the PubMed-Medline platform, using the following keywords: Skin, Mpox, blister.

RESULTS

Micrograph 1A shows a thin human skin biopsy stained with hematoxylin and eosin (H/E). In the upper part, the deep red color highlights the epidermis or stratified squamous epithelium (1) associated with its lamina propria and papillary connective tissue. Under the epithelium, the dermis (2) is visible, appearing light pink and corresponding to dense, irregular connective tissue. The hypodermis (3) is visible toward the bottom, with abundant adipose tissue, cross sections of hair follicles, and blood vessels. Figure 1B shows a thick human skin biopsy highlighting the epidermis (1), the great thickness of the stratum corneum (2), and the width of the dermal papillae (3). Toluidine blue stain (0.1 %).

Micrograph 2A shows a general section of thin human skin, with the epidermis (1) standing at the top. Micrographs 2B and 2C show increasing magnification (40x/100x, respectively). 2B shows the epidermis (1), where the different cell layers of the stratified squamous epithelium can be seen and highlighted: basal (2), intermediate (3), and stratum corneum (4). In 2C, several layers of keratinocytes are observed, closely packed together (1), and separated by a thin, light-colored peripheral line crossed by multiple small lines that connect the cells through desmosomes (2). H/E staining.

Micrograph 3 corresponds to a human skin biopsy showing the epidermis (1) and its dermal papillae (2). A tactile corpuscle (circle) is visible in the dermal papillae. Silver staining.

DISCUSSION

Mpox was first isolated in Denmark in a colony of cynomolgus monkeys from Singapore, which were used for poliovirus research (Daiyab & Daiyab, 2023). Mpox did not significantly impact worldwide when the first cases were reported in Africa. However, this changed when the first non-endemic outbreak outside the continent was reported in the United States in 2003, which was linked to contact with rodents from Guinea, Africa. Until 2018, no new cases were documented in the United States due to travel. Meanwhile, an outbreak emerged in Nigeria, where 122 confirmed or probable cases were documented between September 2017 and September 2018.

The new Mpox outbreak began in May 2022, when several confirmed cases were reported simultaneously in different parts of the world, causing uncertainty about how these cases appeared, as they were unrelated to any contact or visit to a common country (Lum *et al*., 2022).

The infected patients present symptoms such as fever, headache, muscle pain, itching, and discomfort in the affected areas, followed by characteristic skin or mucous membrane lesions (presence of blisters). Four phases have been identified in the blisters: disorganization of the dermo-epidermal junction (basal degeneration, basal layer), loss of cohesion between keratinocytes (desmosomes), observed in pemphigus vulgaris (acantholysis), perivascular

inflammation (dermis), and inflammatory infiltrates (neutrophils, eosinophils, or lymphocytes in the dermis or epidermis, depending on the underlying cause). Intraepidermal lesions and blisters (separation occurs within the epidermis), subepidermal lesions (separation occurs between the epidermis and the dermis), and dermal lesions (may form in the dermis, although they are less common) may also be observed (Karagoz *et al*., 2023).

Three phases are observed in intraepidermal blisters: a) the cells of the epidermis first become edematous and then necrotic, finally detaching; b) the edema separates the epidermal cells (spongiosis); and c) the epidermal cells lose lateral contact with each other due to the rupture of their desmosomes (acantholysis). This restricts access to nutrients, oxygen, and other regulatory factors, which induce mitosis restriction and stimulate cell necrosis (Abijana *et al*., 2011; Abreu-Velez & Howard, 2012.

Unlike other viruses, the Mpox virus replicates in the host cell's cytoplasm. Cell infection can be classified into three steps: adhesion, hemifusion, and entry into the nucleus, which occurs in the cell membrane. Poxviruses produce two distinct types of infectious particles: mature virions and extracellular virions. Molecules such as glycosaminoglycans, which include chondroitin sulfates, heparin, and laminin, help the virus attach to cells, where viral proteins such as D8L, A27L, A34R, A26L, and H3L are essential for the process. After binding, the virus fuses its envelope with the host cell membrane and releases its genetic material into the cell so that RNA polymerase can initiate gene transcription. Viral DNA synthesis also occurs in the cytoplasm, where most virions remain mature intracellular virions. The remaining virions acquire a second envelope that allows them to be transmitted from cell to cell as extracellular virions (Huang *et al*., 2022; Wang *et al*., 2023).

Mpox infection can be self-limiting, as its severity varies depending on the specific viral strain, the individual's immune status, and preexisting complications. Initial symptoms may include fever, pain, fatigue, and lymphadenopathy. After the latent period of the virus (up to two weeks), patients may develop prodromal symptoms such as fever, chills, headache, and myalgia; this is subsequently followed by rashes that evolve from papules to vesicles and pustules, eventually forming scabs that heal, leaving scars. This phase can last 2 to 4 weeks. The virus enters the body through mucous membranes or broken skin, spreading through immune cells resident in tissues and lymph nodes. Understanding the modes of transmission is essential for establishing effective control and prevention measures. Mpox transmission can occur through direct contact with infected animals (zoonotic transmission), person-to-person contact, contact with skin lesions or bodily fluids, aerosol contamination, and has also been reported in conditions of close proximity (Lu *et al*., 2023; OPS, 2023).

The epidemiology of Mpox has undergone significant changes in recent years, especially outbreaks originating in regions not endemic for the virus, suggesting rapid adaptation and increased transmissibility. Virus transmission affects men more than women, with the age group most frequently infected being people between 21 and 50 years of age. Men (70%-98 % of cases) became infected mainly through homosexual and bisexual behavior (30%-60 %). Significant risk factors for adults were sexual and non-sexual contact with people who had Mpox and risky sexual behavior. For children, risk factors were close contact with an Mpox-positive person and previous animal exposure. More than 14,000 cases have recently been reported, with a mortality rate of 3.5 %. On August 14, 2024, the WHO declared Mpox an international health emergency due to a 160 % increase in cases compared to the previous year (Sharif *et al*., 2023; WHO, 2024; Ogoina *et al*., 2024).

In Chile, the Ministry of Health took safety measures, strengthening surveillance in line with international recommendations, consisting of alert notifications, security and surveillance, laboratory capacity for studies and tests, Mpox immunization, and pre-and post-exposure vaccination for specific target groups. During the global Mpox outbreak in 2022, several cases of occupational transmission were reported among healthcare workers, including one case while healthcare personnel was performing procedures to take a skin sample from an HIV-positive patient who also had lesions consistent with Mpox. In this case, the virus incubation period was relatively short, consistent with the nature of percutaneous transmission. In our country, 1,481 cases and three deaths have been diagnosed from June 17, 2022, to June 30, 2024 (WHO, 2024; Zacur, 2023; MINSAL, 2024; ACHS, 2024).

Histopathological examination may be the key to diagnosing Mpox by comparing the changes observed in Mpox-positive biopsies (Bayer-Garner, 2005; Maronese *et al*., 2022). The increase in cases has undoubtedly been evident over time, and further research is needed to understand how the virus persists in nature and to explore the associations between pathogens and hosts and how environmental conditions influence the virus and living beings (Mitjà *et al*.,2023; Karagoz *et al*., 2023).

In this histological study of normal human skin epithelium, the layers and keratinocytes are organized orderly: basal, intermediate, flat, and keratinized layers. In

the intermediate layer, desmosomes are easily observed, occupying the intercellular spaces as fine-colored lines, including several cell layers (Figs. 2A, 2B, 2C). However, human skin biopsies diagnosed with mpox observed by Ortins-Pina *et al*. (2023), describe keratinocyte lesions in the intermediate layers, with cell necrosis, abundant debris, and the presence of the virus in the form of large, strongly acidophilic cytoplasmic inclusion bodies (similar to red blood cells, 7 to 8 μm). Clinically, this region is one of the most affected by the blisters resulting from Mpox infection, and it is where the cytoplasmic inclusion bodies characteristic of the disease would be observed (Ortins-Pina *et al*., 2023).

It is important to highlight that these infections affect human, animal, and ecosystem health. Therefore, authors and national and international health institutions suggest global "one health" policies for the current and future prevention and treatment of emerging outbreaks (Alakunle *et al*., 2024). All results from health and research centers should always be available in easily accessible applications. However, the best protection method is to pay attention to personal hygiene and avoid contact with used objects and animal waste (Karagoz *et al*., 2023). The data provided is essential to remain vigilant about these pandemics or emerging infectious diseases, which often cannot be addressed using high-tech tools due to a lack of financial resources. Therefore, the study or histological analysis of target organs under normal conditions would be an excellent alternative for detecting tissue changes with pathologies early. Awareness regarding these changes, especially in the skin, could be very beneficial for medical and healthcare personnel who treat patients with infected lesions, such as those infected with monkeypox.

These findings indicate that routine histological techniques, such as hematoxylin and eosin staining, could also be a relevant tool for diagnosing and confirming infectious diseases such as Mpox, thereby providing an early indicator of infection and disease.

CONCLUSION

Interactions between viruses, humans, and animals are crucial to understanding transmission dynamics. Mpox is an inappropriate term since the largest reservoir has been found in rodents hunted for consumption. The virus has now been found in numerous species, including monkeys, rats, striped mice, squirrels, and dormice. Undoubtedly, further research is needed to understand how the virus persists in nature and its degree of infectivity. This study highlights the importance of understanding normal histological patterns of the skin and comparing them with alterations caused by viral or other agents. Histological analysis of the skin could be used as a biological marker in the pathogenesis of infectious diseases and their projection in health emergencies, improving the treatment and management of patients and enabling the design of preventive measures to protect infected individuals and medical and healthcare personnel, who are essential figures in our lives. However, the best protection method is to pay attention to personal hygiene, avoid touching used objects, and avoid animal waste.

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RESUMEN: La piel, el órgano más grande del cuerpo, es también una barrera protectora eficaz. Está compuesta por la epidermis, la dermis y la hipodermis. Sin embargo, también constituye una puerta de entrada para infecciones que provocan degeneración localizada en sus componentes. El conocimiento de los patrones histológicos estándar es fundamental para comprender los cambios observados debido a la acción de agentes infecciosos emergentes, como es el caso de la viruela del mono (Mpox) (WHO, 2024). Este estudio tuvo como objetivo comparar los cambios histológicos entre la piel sana y aquellos descritos en la literatura en piel infectada con Mpox, con el fin de diseñar mejores políticas preventivas para la salud pública. Se realizaron biopsias de piel humana sana, obtenidas de material docente de la Universidad de Chile, y se sometieron a técnicas histológicas de rutina. Los resultados muestran una estructura epidérmica típica con epitelio escamoso estratificado, una dermis compuesta por tejido conectivo denso irregular y queratinocitos, y una hipodermis con abundante tejido adiposo y vasos sanguíneos, observándose corpúsculos tactiles en las papilas dérmicas. La comparación entre patrones histológicos normales y alterados en la piel podría ser una herramienta útil para diagnosticar y confirmar patologías infecciosas como el Mpox entre otras, y así obtener indicadores precoces de infección y enfermedad. No obstante, en el caso de patologías infecciosas, la mejor medida de protección es mantener una buena higiene personal, evitar el contacto con objetos usados y con excrementos de animales.

PALABRAS CLAVE: Patologías Emergentes; Salud Pública; Histología Cutánea; Marcadores Biológicos.

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