Cytopathological Diagnosis of Herpes Simplex Viral Mastitis: Three Rare Cases and a Review of the Literature

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Abstract

Context: Virus infection has a unique morphological change in cell pathology. It is also of great significance in the diagnosis of herpes simplex mastitis.

Aims: To evaluate the practicability and feasibility of cytological diagnosis of herpes simplex virus mastitis.

Materials and Methods: Three cases of HSV mastitis diagnosed by cytology smears were collected. The papules were stained with Wright-Giemsa to observe the morphological characteristics of the cells.

Results: In the background of inflammatory cells in the smear, the cells with characteristic changes can be seen in the mass and scattered distribution. The nuclear size increased significantly, and mononuclear, binuclear, trinuclear and multinuclear nuclei appeared. The nuclei of multinuclear were crowded and mosaic. The chromatin of nucleus changed like frosted glass, and the chromatin became thicker at the edge of nucleus. Inclusion bodies were found in the nucleus, eosinophilic, with different sizes and irregular shapes. These characteristics are helpful to distinguish from other lesions.

Conclusion: Scraper cytology is a diagnostic tool with simple operation, small trauma and pain, and it has important application value in the differential diagnosis of HSV infection, which deserves the attention of clinicians.

Keywords: Cytopathology, herpes simplex virus, mastitis, nipple

Introduction

Herpes simplex virus (HSV) easily invades human skin or mucosa[1] and has two serotypes, hsv-1 and hsv-2. HSV infection of mammary nipple or areola is relatively rare, and the infection is more common in lactating women, mainly caused by the injury of nipple skin and mucosa through breastfeeding. Herpes simplex mastitis without lactation is quite rare. We report three cases of herpes simplex mastitis and review the literature.

Materials and Methods

Clinical features

A retrospective analysis was conducted on three cases of herpes simplex mastitis diagnosed by cytopathology, all of which were from the Department of Pathology, the Second Clinical College of Yangtze University, from January 2015 to June 2019. All the patients were female, including two cases of lactation, one case of nonlactation, one case of the bilateral nipple during lactation, and two cases of the unilateral nipple (one case of left and right nipple). The main symptom of the patient was nipple pruritus and pain. Physical examination showed erosion, exudation, and scab on the surface of the nipple, with a course of 7 days to 2 months. Early clinicians treated it as allergic dermatitis, eczema, and other diseases, but the treatment was ineffective. After cytological examination, it was diagnosed as HSV infection. Oral antiviral drugs and local use of antiviral ointment cured it, leaving no scars. The study was approved by the ethics committee at the hospital, and informed consent was obtained before. Table 1 for details.

Cytopathological examination

Sampling method

The scrape® method was adopted for sample collection. One corner of the sterilized slide can be used to peel off...
The scab on the lesion surface, so that the bright red wound surface can be exposed. At this time, there may be a small amount of infiltration or bleeding, then another corner of the sterilized slide can be used to quickly scrape the fresh bright red wound surface; micron-like tissue is usually obtained. The scraped sample was transferred into the ordinary slide, pushed away with appropriate pressure, air-dried and fixed, and Wright-Giemsa staining was performed.

**Staining method**

Different from other literatures (OMIT), Wright-Giemsa staining was adopted, which takes into account the advantages of Wright staining and Giemsa staining and can better display the characteristics of the nucleus and cytoplasm. After air drying, Wright-Giemsa dye was added and fixed for 10 s, then phosphate buffer (pH 7.0) was added and stained for 10 min. In the end, the smear was rinsed with flowing water, followed by microscopic observation and cytopathological diagnosis.

**Results**

**Cytopathologic features**

On the smear, there were some inflammatory cells (mainly neutrophils, with a small number of lymphocytes and histiocytes) and scattered epithelial cells. More atypical cells were seen, in clumps and scattered, and the morphology of these cells had characteristic changes. The size of these cells increased significantly, especially the nucleus. Mononuclear, dikaryotic, trinuclear, and multinucleated nuclei appear [Figure 1]. Some nuclei of multinucleated cells were lined up like private soldiers [Figure 2]. Cells with multiple nuclei show crowded nuclei and mosaic pattern [Figure 3]. Nuclear chromatin changes like ground glass and chromat thinning at the edges of the nucleus. Inclusion bodies are seen in the nucleus; they are eosinophilic, unequal in size, and irregular in shape. Some of the intranuclear inclusions occupy the entire nucleus. Around the nucleus, bright halos or transparent areas appear [Figure 4]. In Wright-Giemsa staining, the cytoplasm showed strong basophilic staining.

**Cytopathologic diagnosis**

Based on these characteristics, cytology can make a diagnosis consistent with HSV infection.

**Discussion**

HSV mastitis is relatively rare and the infection route of the nipple is mainly contact transmission, which can be transmitted through saliva or intimate contact.[12-14] Most of the infected are lactating women, and the main reason is that the nipple skin and mucosa are infected with HSV through breastfeeding.[5] However, nonlactating nipple HSV infection is very rare and rarely reported in the literature. When the nipple is infected, the clinical characteristics are usually rapid onset and short course of disease, generally occurring in the unilateral breast[6,7] and rarely in the bilateral breast.[6,7] The lesions are usually confined to the nipple and areola.[11]

The small blisters may appear on the skin in the early stages; when the blisters burst, the skin becomes ulcerated, red, swollen, and painful. Later, there is nipple erosion and scab, with itching and pain. These clinical features are easily confused with other breast inflammatory lesions, such as bacterial infection, dermatomycosis, atopic dermatitis, allergies, eczema, and other diseases.[11] HSV infection is usually HSV-1, but virological identification is often difficult to perform in practice. Although HSV infections are usually self-limited, healing takes about 7–10 days, patients are often more painful because of the abundance of nerve in the nipple and areola, which is also related to the neurophilic nature of the virus.
Finding an effective, simple, and inexpensive diagnostic method is very important for establishing the diagnosis of the disease as soon as possible. Literature has shown that cytology is a simple, rapid, cheap, and reliable method for the diagnosis of skin tumors, immune bullous lesions, infectious diseases, and hereditary skin diseases.[15] In the case we reported, after cytological diagnosis, acyclovir was taken orally and antiviral ointment was applied locally, which quickly relieved the patient’s clinical pain. After a few days, the patient completely healed without a scar. It is also a warning to clinicians to consider the possibility of HSV infection when nipple inflammation does not respond to routine antibiotics.

When HSV is infected, its main cytopathological feature is the visible nuclear changes in the infected cells. Nuclear changes include, for example, balloon-like denaturation, a nucleus with a uniform ground-glass appearance, an eosinophilic inclusion body, and multicellular mosaic nuclei.[14,16] In addition to the cytopathological cases of HSV infection at the breast (nipple secretion smears), the diagnosis of HSV mastitis by nipple secretion has also been reported in the literature. Kobayashi[17] described cytological changes in nipple secretion smears in two patients with HSV infection. In their cases, cytologic examination of a smear of milk secretion showed the glassy appearance of polynuclear syncytial cells. This also suggests that cell morphology is a quick and valuable tool for the detection and eventual confirmation of HSV infection.

In the cases we reported, in this way, the sampling was performed using the more sharp corners of sterile slides, which was only a mildly traumatic process. It is easy to remove attachments on the lesion surface; more micron-like tissue was obtained. Scraping and smears require some professional skill. Because of this sampling method, cytological results may be more sensitive. In our case, Wright-Giemsa composite staining was better able to show the cytological characteristics of HSV infection.

For viral infections, the gold standard for diagnosis is usually isolation and culture. However, for HSV infection of the nipple, after the acute stage of skin injury, the skin will scab and secretions will decrease, then the success rate of virus separation is low. At this time, serum HSV antibody was detected by serological test in the laboratory, which could reflect the infection status of HSV. Once the HSV immunoglobulin M antibody was detected in the serum, HSV infection was indicated recently. However, the serological test results reflect the recent infection of the whole body, which cannot reflect the local organ infection. Scraping cytology just reflects the state of the local organ infection, which can provide a more accurate diagnosis for HSV infection.

Cell morphology of breast HSV infection needs to be differentiated with the following lesions: (1) Paget disease of the breast, clinically can also be manifested as the erosion of the surface skin of the nipple and scabbed lesions, can involve the areola or even the entire nipple, naked eye performance
of the two is not easy to distinguish, but Paget disease course is usually longer. However, the cell morphology of Paget’s disease of the breast is quite different from that of HSV. Scattered and small nests of tumor cells can be seen in the epidermis. The cell volume is large, round or oval, and the nuclear atypia is obvious,[18−20] lacking the characteristic cellular changes of HSV infection. (2) Herpes zoster virus infection at the nipple, usually latent in the nerve root or ganglion, when the virus is activated, through the sensory nerve fiber axon down to the dominant skin area. At the beginning of the course of the disease, there was abnormal sensation and significant pain in the local skin, followed by red rash and herpetic vesicle, which were mostly seen in the trunk and the face area. It was very rare to involve the breast mucosa,[21] which was mainly differentiated from HSV infection according to clinical manifestations. Rapid DNA detection can also be performed if conditions permit.[22] (3) Human papillomavirus infection of the mammary gland: it is relatively rare clinically, with characteristic concave empty cells visible in morphology, and it is often associated with human papillomavirus infection of the lower genital tract, which is easy to distinguish from HSV infection.[22] (4) Eczematous lesion of the nipple: it is an inflammatory lesion of unknown cause, which is characterized by erosion and itching of the nipple skin surface, often with serous exudate and thin scab. Microscopically, a background of inflammation is seen, with varying degrees of squamous hyperplasia,[23] no atypia of nuclei, and sometimes diffuse keratinized squamous cells, and scattered eosinophils.

**CONCLUSION**

Nipple HSV infection of the breast is difficult to be diagnosed due to the lack of specific changes in its clinical manifestations. However, scrape cytology is a diagnostic tool with simple operation, small trauma, and pain, and it has important application value in the differential diagnosis of HSV infection, which deserves the attention of clinicians.

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**Conflicts of interest**

There are no conflicts of interest.

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