

# Microscopic Nikolsky's Sign: Is It Useful for Diagnosis of Pemphigus Vulgaris?

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## Abstract

**Background:** Pemphigus vulgaris (PV) is an autoimmune blistering disease, caused by autoantibodies against desmoglein (Dsg) 3 and/or Dsg1 which induce the loss of adhesion between keratinocytes. Nikolsky's sign is the ability to induce peripheral extension of a blister as a consequence of applying lateral pressure to the border of an intact blister. If the weakening of the intercellular adhesion is present but not marked, then the damage may be demonstrated only microscopically (microscopic Nikolsky's sign and can increase the sensitivity of the histopathological studies.

**Methods:** We studied 40 patients and divided them randomly into two groups (A, B). Group A were subjected to the tangential pressure over the perilesional skin before a biopsy specimen was taken from that site; group B patients were subjected to a biopsy without the tangential pressure technique.

**Results:** Histopathological changes of pemphigus vulgaris were present in 30% of the patients in group A and 5% of the patients in group B. They were not statistically different. The presence of microscopic Nikolsky's sign was significantly higher in patients with generalized disease.

**Conclusion:** Microscopic Nikolsky sign can increase the sensitivity of histologic diagnosis of PV. (*Iran J Dermatol* 2008;11: 64-66)

**Keywords:** pemphigus, diagnosis, histology

## Introduction

Pemphigus is a chronic autoimmune disease, characterized by the loss of cell-cell adhesion, i.e., acantholysis, in both mucous membranes and epidermis, resulting in the formation of mucocutaneous blisters and erosions. The circulating autoantibodies against desmoglein (Dsg) 3 and/or Dsg1 induce the loss of adhesion between keratinocytes and subsequent blister formation.<sup>1-3</sup>

Formation of blisters can be elicited in pemphigus patients with active blistering by applying lateral pressure to normal-appearing skin at the periphery of active lesions. The result is a shearing away of the epidermis in normal appearing areas, a phenomenon known as the Nikolsky's sign<sup>4-7</sup>.

It was first described by Piotr Vasiliyevich Nikolsky (1858-1940) who was a Russian dermatologist.<sup>4,5</sup> It is characteristically seen in intraepidermal bullous disorders, whereas in subepidermal vesiculobullous diseases the sign is generally absent. Besides the clinical manifestations, routine laboratory examinations for diagnostic

purposes are the standard histology, direct immunofluorescence (DIF), indirect immunofluorescence (IIF), and ELISA assays.<sup>2</sup>

However, PV can start slowly and the vesicles and bullas may rupture soon, and may become secondarily infected. Thus histopathological studies are not always helpful and indeed, immunofluorescence studies may not be available in some centers.

When the pathological changes are induced after applying tearing tangential pressure to skin, it is defined as microscopic Nikolsky's sign.<sup>8</sup>

It has been proposed that microscopic Nikolsky's sign may be a better and more sensitive method of rapid diagnosis and can increase the sensitivity of the histopathological studies.<sup>8</sup> Here we tried to assess the usefulness of microscopic Nikolsky's sign in a randomly selected group of patients with PV.

## Patients and Methods

Forty patients (11 males, 29 females) admitted in Razi hospital, Tehran with definite PV were selected over a 12-month period. The study only included

the patients with cutaneous lesions and pemphigus patients with only mucosal lesions were not included. The diagnoses were based on the clinical manifestations and confirmed by histopathology, and direct immunofluorescence studies in all cases. Patients with clinically positive Nikolsky's sign were excluded. Clinical Nikolsky's sign was positive if clinically visible epidermal detachment and/or erosion formation was achieved after the tangential pressure (explained below). Patients who received treatment regimens from 30 days before the admission were also excluded.

A questionnaire containing the patient profile and disease activity scores were completed by the investigator at the time of admission which bore the following data: name, age, gender, group (A or B explained below), interval between the disease onset and the time of admission (months), number and sites of the mucosal and cutaneous lesions, surface area of the involved skin, number of new lesions per day in the past week, presence of generalized disease defined as involvement of  $\geq 4$  of skin area and  $\geq 2$  of mucosal surface area, the site of the biopsy and the peripheral lesional extension, defined as the extension of an intact bulla after a vertical pressure on the top of it and scored mild if  $< 0.5$  cm; moderate if 0.5-2 cm and severe if  $> 2$  cm.

Patients were divided randomly into two groups: group A (n=20) and group B (n=20). Clinically normal peri-lesional skin was selected at about two centimeters apart from a relatively new and non-healed bulla or erosion. For the patients in group A, firm tangential pressure (Nikolsky's maneuver) was applied using the index finger, first in one direction for 10 seconds and then at right angles to it, for another 10 seconds. This maneuver was done twice for each patient at the same site and then a biopsy was taken. The tangential pressure technique was always applied by the same investigator. A 3.5-mm disposable biopsy punch was used to take the biopsy specimen. Group B patients were similarly biopsied but without the tangential pressure. Specimens were placed in 10 % formalin and sent to the pathology lab for paraffin embedding and staining with hematoxylin and eosin (H&E). Sections then viewed with a light microscope by three pathologists and the findings were recorded. Processing of biopsy specimens was the same in all cases. Presence of epidermal cleft, acantholysis, inflammation and spongiosis were recorded but only the first two items were mentioned as positive pathologic changes in this study.

## Results

A total of 45 patients were evaluated for the presence of clinical Nikolsky's sign. Five patients were positive (11.1%) who were further excluded from the study.

Forty patients (29 females, 11 males) were included in the study. The mean duration between the onset of the disease and the time of admission (delay time) was 6.7 months. The mean age of the patients was 50.7 years which did not differ statistically between group "A" (50.2 y.) and group "B" (51.3 y).

Thirty three patients (82.5%) had mucocutaneous disease while 7 (17.5%) had only cutaneous lesions. As we mentioned before, the patients who only had mucosal PV were excluded. Seventy percent of the patients in general, and 84.8% of the patients with mucosal lesions had involvement of the buccal mucosa. For the palatal, labial, lingual, genital, nasal, laryngeal and gingival mucosa, the percentage of affected patients was 50%, 47.5%, 22.5%, 20%, 10%, 5% and 5%, respectively.

Twenty seven patients (67.5%) had bulla and erosions on their backs, 22 patients (55%) on their chests, 21 patients (52.5%) on their scalps and 21 patients (52.5%) on their abdomens including the umbilicus. The upper limbs (including the axilla), face, buttocks and lower limbs were affected in 15(37.5%), 9(22.5%), 7(17.5%) and 7(17.5%) of the patients, respectively. Generalized disease was present in 27.5% of the patients. (Table 1)

The total of 7 patients (17.5%) developed the microscopic Nikolsky's sign. After the tangential pressure, 30% of the patients in group A and 5% of the patients in group B developed the positive pathological changes. In addition, 5 patients (12.5%), had spongiosis (3 in group A and 2 in group B) and 5% of the patients who were both in group A had infiltration of inflammatory cells in the viewed sections but were not referred to as positive signs. There was no significant statistical relationship ( $p=0.095$ ) between the skin rubbing with tangential pressure (Nikolsky's maneuver) and development of positive pathological changes (Nikolsky's signs).

In patients with a generalized disease, the probability of the positive microscopic Nikolsky's signs was significantly elevated ( $p=0.011$ ). However, involvement of the other mucosal and cutaneous areas did not show such an association.

The changes were also not related to sex, age, extension of the lesions, and the rate of development of new lesions per day.

## Discussion

Nikolsky's sign is a valuable clinical finding in differentiating bullous dermatoses. Its presence is an indicator of active acantholysis and altered structural integrity within the dermis. Nikolsky's sign is classically associated with pemphigus vulgaris. However, other blistering conditions are also known to exhibit this sign including pemphigus foliaceus, bullous pemphigoid, epidermolysis bullosa, staphylococcal scalded-skin syndrome (SSSS), and toxic epidermal necrolysis (TEN)<sup>9,10</sup>

Although the Nikolsky's sign is highly specific, it only offers moderate sensitivity for the diagnosis of PV.<sup>11</sup> The sensitivity of direct Nikolsky's sign (performing the test on the normal skin away from the bulla) is predicted to be about 38% for the diagnosis of pemphigus.<sup>11</sup> Our study resulted in a frequency of 11.1 % for positive clinical Nikolsky's sign in PV.

Low sensitivity of clinical Nikolsky's sign and the limitations of sophisticated laboratory tests make the diagnosis of PV difficult at least in some patients.

It has been proposed that microscopic Nikolsky's sign may be a better and more sensitive method of rapid diagnosis and can increase the yield of the histopathological studies.<sup>8</sup>

Lateral pressure applied by the index finger provides the shearing force to disrupt the intercellular adhesion in clinical Nikolsky's sign. If the weakening of the intercellular adhesion is present but not marked, then the same shearing force may produce minimal damage at the cellular level which can be demonstrated only microscopically. As microscopic Nikolsky's sign sometimes spans only a few cells, serial sections may be required to avoid missing a cleavage.

In a study conducted by Hameed and Khan<sup>8</sup>, microscopic Nikolsky's sign was present in 73.9% of the patients who were subjected to tangential pressure over the peri-lesional skin before biopsy compared to 28.6% of those who were biopsied without any manual lateral pressure to the skin. They also reported an absence of inflammatory components in the cleft and the dermis below. In our study, only 30% of PV cases developed microscopic signs of acantholysis and cleft formation after tangential pressure. Inflammatory infiltrates were also rare in our pathologic sections. This finding infers that the presence of acantholysis and intraepidermal cleft were due to the shearing forces applied mechanically, but not the result of inflammatory processes.

We demonstrated a statistically significant relationship between the development of pathological changes and the presence of generalized form of the disease.

PV patients with mucocutaneous involvement have both Dsg3 and Dsg1 antibodies.<sup>12,13</sup> Presence of the generalized disease is probably due to much higher pemphigus antibody levels, making the development of microscopic Nikolsky's sign more frequent, as we defined here.

This study showed that the microscopic Nikolsky's sign is not as sensitive as mentioned in the original report.<sup>8</sup> However, in those areas where facilities for immunofluorescence are limited and appropriate lesions for obtaining meaningful results by routine histopathology are not readily available, especially in patients with generalized PV, microscopic Nikolsky's sign could be used as an adjunctive diagnostic measure.

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