Acta Derm Venereol (Stockh) 1997; 77: 319–322

Is Vulvar Vestibulitis an Inflammatory Condition? A Comparison of Histological Findings in Affected and Healthy Women

ELISABET NYLANDER LUNDQVIST[1], PER-ÅKE HOFER[1,2], JAN I. OLOFSSON[3] and INGA SJÖBERG[3]

Departments of [1]Dermatology and Venereology, [2]Pathology and [3]Obstetrics and Gynecology, Umeå University Hospital, Sweden

Vulvar vestibulitis, as defined by Friedrich, is considered to be inflammatory, despite the fact that the normal histology of this specific area has previously not been characterized. The aim of the present study was to compare the normal histology of the vulvar vestibulum with findings in localized vulvar vestibulitis. Biopsies were taken at the area of the vestibulitis, i.e. at the openings of the Bartholin's duct. Eleven control specimens were examined histologically and compared to 24 specimens obtained from 20 patients. All samples were also tested for human papillomavirus, and they were all negative.

In control specimens, as well as in specimens from patients, subepithelial inflammatory cells, sometimes aggregated into lymph follicles and/or small groups of lymphocytes were found. The conclusion is reached that the occurrence of inflammatory cells in vestibular tissue is a normal finding and cannot serve as a histological indicator of vulvar vestibulitis. ***Key words: lymph follicles; inflammatory cells; opening of vulvar Bartholin's duct; human papillomavirus.***

(Accepted February 10, 1997.)

Acta Derm Venereol (Stockh) 1997; 77: 319–322.

Elisabet Nylander Lundqvist, Department of Dermatology and Venereology, University Hospital, S-901 85 Umeå, Sweden.

Vulvar vestibulitis, originally described by Skene in 1889 (1), remains a challenge to affected women and to their physicians (2–4). The definition of vulvar vestibulitis, according to Friedrich (5), is severe pain on vestibular touch or attempted vaginal entry, tenderness to pressure with cottontips within the vulvar vestibule, and vestibular erythema of various degrees. The symptoms have a duration of more than 6 months. Mostly younger women are affected.

Localized vestibulitis is characterized by a small area of erythema and intense tenderness around the openings of the ducts of the Bartholin's glands. Embryologically, the vestibule represents the only portion of the female genital tract that is of endodermal origin (6).

Vulvar vestibulitis is stated to be due to/involve an inflammation of unknown origin (2, 4, 7–10), although the normal histology has not previously been described. At least some of the earlier reports consisted mainly of patients with fairly high mean ages (4, 7, 11). Typically, control groups were not available (4, 9, 12), with the single exception of one report mentioning the macroscopically normal margins of tissue from patients diagnosed as having other conditions (8). In elderly patients, vulvar vestibulitis was rarely associated with human papillomavirus (HPV) infection (7), but it is unclear whether that holds true for younger patients.

Our present report describes the microscopical findings in the area affected by localized vulvar vestibulitis in patients fulfilling Friedrich's criteria as well as in healthy volunteers. The possible relationship to HPV was also studied.

MATERIAL AND METHODS

Twenty patients, aged 20–29 years, who all fulfilled Friedrich's criteria for vulvar vestibulitis, were enrolled in the study. They were compared to 11 healthy volunteers, aged 18–25 years. The study was approved by the Ethics Committe at Umeå University. None of the volunteers had any history or symptoms or signs of vulvar disease. Informed consent was given. After application of lidocaine plus prilocaine as a local anaesthetic cream, a punch biopsy with a diameter of 4 mm was taken at the opening of Bartholin's gland on the right side, except for 2 patients where it was taken on the left side, and 4 patients where it was taken bilaterally. The biopsies were immediately fixed in 4% buffered formaldehyde, paraffin-embedded and cut into 5-mm sections. The sections were stained with haematoxylin-eosin, van Gieson's method, and the PAS (periodic acid-Schiff) technique, evaluated in a light microscope and photographed.

Detection of HPV using polymerase chain reaction (PCR) analysis

Thirteen tissue specimens from patients with vestibulitis, 9 specimens from healthy controls and 6 cervical biopsies from patients with previously diagnosed cervical HPV infection, used as positive controls, were homogenized in lysis buffer (0.32 M sucrose, 10 mM Tris-HCl, 5 mM $MgCl_2$ in the presence of 1% Triton X-100), pelleted and rinsed twice in 10 mM Tris-HCl (pH 8.0). After digestion for 60 min at 60°C, and 37°C for 14–16 h with proteinase K in the presence of 0.032% NP40 and 0.032% Tween 20, samples were treated with RNAse A for 1 h at 37°C and DNA extracted using phenol-chloroform. A two-step nested PCR amplification protocol of HPV genome from approximately 1.5 mg DNA was used, exactly as previously described (13). All specimens were also amplified with the β-globin primers PCO3 and PCO4, according to Saiki et al. (14) to exclude false negative results. For detection, samples were electrophoresed through a 1% agarose gel and stained with ethidium bromide.

RESULTS

Surface epithelium

The biopsies from both controls and patients were in general covered by a non-keratinized stratified squamous epithelium. One control specimen had a small area covered by stratified cylindrical epithelium, which seemed to extend from duct epithelium, and 4 patient specimens were partially or totally covered by a similar type or the same type of epithelium.

Specimens covered by squamous epithelium often had an intermediate stratum with perinuclear clearing in the epithelial cells. This zone varied in expression from well developed to missing. The surface epithelium in both patient and control specimens had an average thickness of about 0.2 mm above rete ridges, and about 0.1 mm above the papillae, and could be as thin as 0.02 mm above more dense collections of inflammatory cells. There was no apparent parakeratosis.

Glands

Mucinous glands were often seen in both control (7/11) and patient (15/24) specimens. These glands could be located

 ISSN 0001-5555

directly under the surface epithelium of the vestibule or ended in glandular ducts. The number of glandular lumina ranged from 1–28 (mean 9; medium 11.6) in control specimens, and from 1–54 (mean 12; medium 14.1) in patient specimens.

Glandular ducts

Ducts or invaginations, mostly with adjacent glands, were seen both in control and patient specimens (8/11 control specimens; 13/24 patient specimens; 12/20 patients). The size of the ducts varied up to a length of slightly more than 2 mm. The three largest ducts, all in control specimens, were probably Bartholin's ducts. Two were lined with squamous epithelium, with none or one occasional gland; one had transitional cell-like epithelium with glands in and around the wall. Some of the invaginations, all covered by surface epithelium, may also represent the opening of a Bartholin's duct. Smaller ducts with a lining of squamous epithelium, which could have cuboidal luminal cells, and with surrounding glands, were seen in 4 biopsies. Possibly, these ducts were also of Bartholin's type. Ducts with pseudostratified cylindrical epithelium, probably minor vestibular gland ducts, were seen in 3 control specimens and one patient specimen. In another vestibulitis specimen the length-sectioned duct had pseudostratified cylindrical epithelium in its deepest part, successively changing to squamous epithelium in the most superficial part.

Stroma

The stroma, consisting of thin collagenous fibres, had a large content of thin-walled vessels. The stroma was loose both in controls and patients. In some specimens, both from controls and patients, there was an adjacent area with more densely packed thicker collagenous fibres, containing more thick-walled, larger and irregularly shaped vessels.

Inflammatory cells

In 3 of the normal biopsies there were altogether four superficial lymph follicles (Fig. 1), covered by a thin epithelium, and with at least three of them adjacent to a duct opening or invagination, and with a germinal center in each of the two lymph follicles in one case. Concerning vestibulitis, 2 specimens had one lymph follicle each, with a germinal centre in both of them. Additional tips of papillae could have densely packed lymphocytes, comprising areas smaller than lymph follicles (Fig. 2). When collections of at least 50 lymphocytes were considered, there were one–two such collection(s) in 4 control specimens (3 of them also contained lymph follicles), and in 12 patient specimens (not in those with lymph follicles), some of them only in occasional sections. Some of these collections were seen adjacent to duct openings. Otherwise there could, immediately under the superficial epithelium, diffusely occur a small to moderate number of inflammatory cells, mainly lymphocytes, frequently with some admixture of plasma cells, and with a similar appearance in specimens from patients and controls.

Minimal or no inflammation was seen in 3 control specimens, and minimal inflammation was seen in 3 specimens from patients (Fig. 3). Abundant inflammation was seen in only 4 patient specimens, all exhibiting a subepithelial band-like inflammation with lymphocytes and many plasma cells (Fig. 4), in 3 of them also lymphocyte collections, and in one a lymph follicle. Epithelial changes or clinical findings characteristic of vulvitis plasmacellularis were lacking.

Adjacent to ducts and glands there could be a small number of inflammatory cells both in controls and patients, but only exceptionally was there a more abundant occurrence of such cells, exemplified by a control specimen with a high percentage

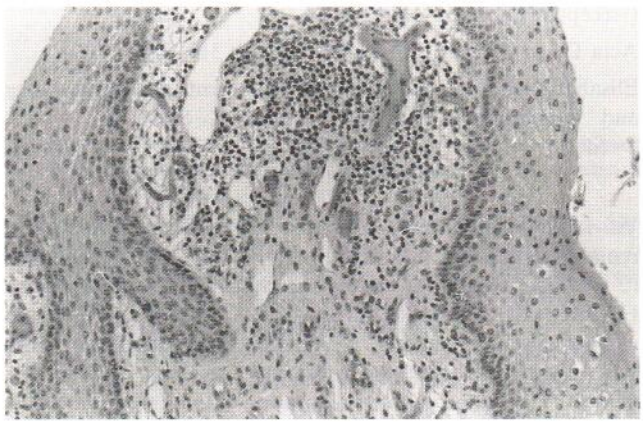

Fig. 2. Patient specimen with collection of more than 50 lymphocytes in a papilla (haematoxylin-eosin, ×242).

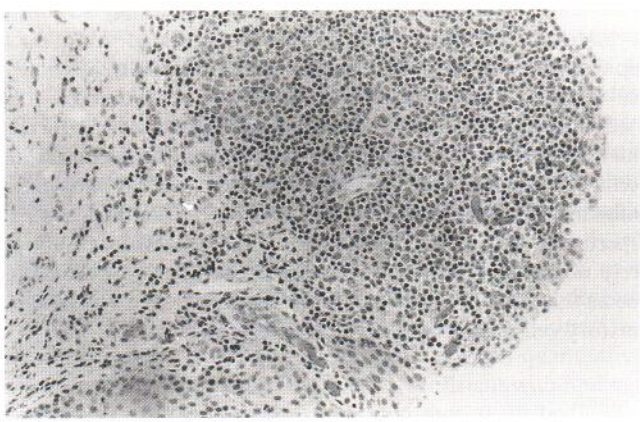

Fig. 1. Control specimen with lymph follicle (haematoxylin-eosin, ×242).

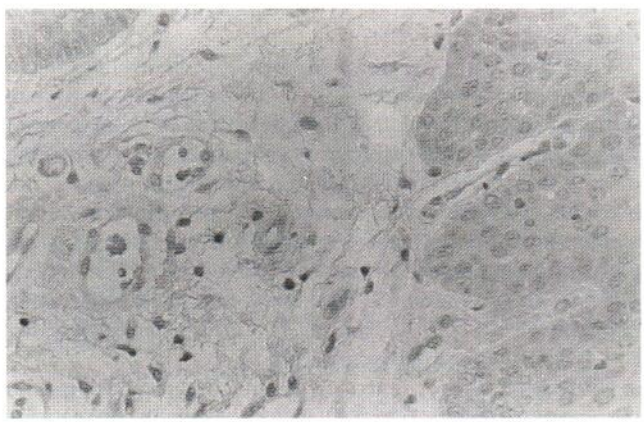

Fig. 3. Patient specimen with minimal inflammation (haemato-xylin-eosin, ×489).

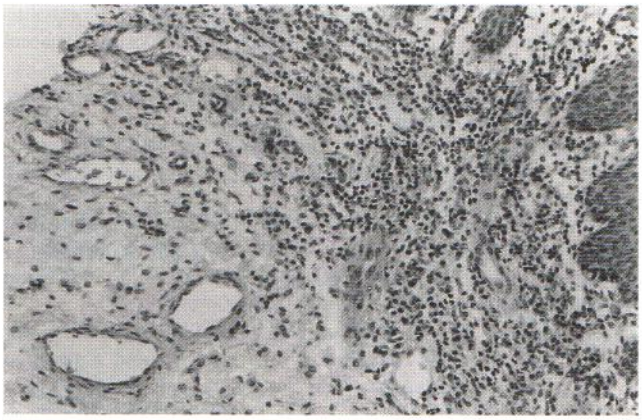

Fig. 4. Patient specimen with subepithelial band-like inflammation (haematoxylin-eosin, ×242).

of plasma cells in the inflammation surrounding a minor vestibular gland duct.

PCR analysis of HPV DNA

All samples obtained from patients diagnosed with vestibulitis and healthy controls tested negative for HPV, using a highly sensitive PCR method (Fig. 5). Notably, all samples tested positive for the β-globin gene, thereby excluding the possibility of insufficient DNA quality and, furthermore, in all samples from positive controls the successful amplification of HPV-DNA was ensured.

DISCUSSION

To our knowledge, the present report of histological findings in vulvar vestibulitis is the first that includes normal control specimens. Furthermore, in our study, only patients with vestibulitis confined to the openings of the Bartholin's ducts were included. In our experience, as well as that of others (15), this is the most common variant of vestibulitis. Whether vestibulitis confined to the openings of the Bartholin's ducts represents a variant or an early stage of more extensive lesions, or whether it is a subgroup of the disease, remains unclear.

In an earlier study Friedrich (16) described a development from an early stage with erythema surrounding the duct openings to a more extensive, diffuse, tender erythema, involving a considerable portion of the vestibule although beginning at the minor gland openings. Our patients with erythema surrounding the Bartholin duct openings did not exhibit such stages of development; however, such differences between various patient materials may represent different interpretations of the clinical findings (17).

In previous reports concerning histological findings in vulvar vestibulitis the mean age, if indicated, is higher than in our material. Apart from studies describing the findings in specimens from vestibulectomy, two reports describe the histological findings at the opening of Bartholin's duct (18) or in the adjacent erythematous area (15). However, these patients had a considerably higher mean age and some of them also had pain sites located away from the openings of the Bartholin ducts.

Parakeratosis and/or hyperkeratosis (4, 7) around the Bartholin's duct opening has so far not been observed to occur normally. Previously in the vestibulum, a lining with non-keratinized squamous epithelium with perinuclear clearing in the more superficial cells has usually been described (7, 19). However, our results indicate that cells with perinuclear clearing may be lacking, and occasionally the surface cells may be cylindrical or cuboidal, with or without a supporting squamous epithelium. Although deviations from the usual vestibular surface epithelium were seen more often in biopsies from vestibulitis compared with controls, it was not a specific or typical finding for vestibulitis. Ducts with squamous epithelium were also found in normal tissue and are not typical of vestibulitis (cf. 7). So-called "vestibular clefts" (8) may also occur normally. In our material, the Bartholin's ducts had a relatively thick epithelial lining, findings which differ from those of Michlevitz et al. (18), where only a single layer of cylindrical cells was depicted.

Most fascinating of all is the finding of inflammatory cells in healthy control specimens, questioning the terminology of what so far has been described as vulvar vestibulitis. This is, to our knowledge, the first report of the occurrence of lymphoid tissue in normal vestibulum. Lymphoid follicles were found more often in controls than in vestibulitis but have only exceptionally been reported previously (4). Whether the condensation of lymphocytes in vestibulitis represents an incidental or immunologically relevant finding remains unclear.

The occurrence of a subepithelial band of lymphocytes with some admixture of plasma cells was not found specific for vestibulitis, as also described elsewhere (17, 18).

The aetiology of the erythema and pain around the opening of the Bartholin's duct remains enigmatic. No obvious infectious agent has been reported. Recent studies have indicated that HPV may be an aetiological factor (11, 19). However, our demonstration that no HPV-DNA could be detected excludes this possibility, a finding in accordance with some recent reports (4, 7, 15).

We conclude that it is not possible to tell whether a biopsy

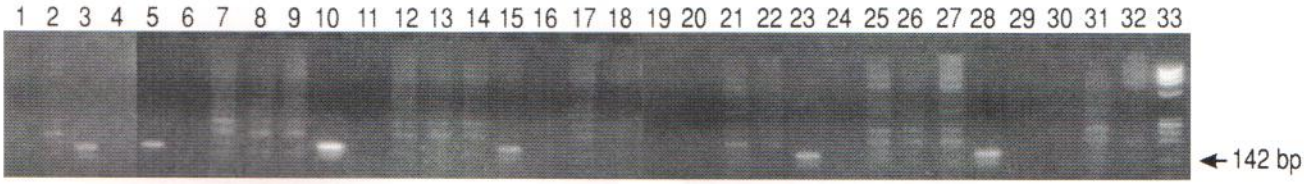

Fig. 5. Polaroid of amplified DNA specimens obtained through biopsies from vestibulitis patients (*lanes 1, 2, 9, 14, 17, 18, 20–22, 25–27, 32*) or from healthy women (*lanes 7, 8, 12, 13, 30, 31*) or positive control samples (*lanes 3, 5, 10, 15, 23, and 28*), respectively. DNA was amplified using a two-step PCR (20 cycles with first primer-pair followed by 30 cycles with second primer-pair) for detection of HPV-DNA, and samples were electrophoresed through 1% agarose gels. *Lanes 4,6,11,16,19,24 and 29*, respectively, are negative PCR controls, and *lane 33* is a DNA molecular size marker (f 174, Boehringer). The size of the expected PCR product is indicated to the right (142 bp).

is taken from a healthy control or from a patient with vulvar vestibulitis. The aetiological course appears not to be related to HPV and still remains elusive.

ACKNOWLEDGEMENTS

The technical assistance of Ms Britta Lindgren and Ms Monica Isaksson is gratefully acknowledged. Financial support was given by the Welander/Finsen Foundation; Samverkansnämnden i Norra Sverige; a scholarship from the Swedish Medical Research Council (to JIO), Novo Nordisk Foundation and the Swedish Society of Medicine.

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