

THE ROLE OF B LYMPHOCYTES AND PRESENCE OF GERMINAL CENTERS IN PATIENTS WITH LOCALIZED PROVOKED VULVODYNIA

LOKALİZE PROVOKE VULVODİNİ HASTALARINDA B LENFOSİTLERİN VE GERMİNAL MERKEZ VARLIĞININ ROLÜ

İrem DEMİRAL KELEŞ¹ , Cenk YAŞA² , Semen ÖNDER³ , Ekrem YAVUZ³ , Sıtkı TUZLALI³ , Süleyman Engin AKHAN² 

¹29 Mayıs State Hospital, Department of Obstetrics and Gynecology, Ankara, Turkey

²Istanbul University, Istanbul Faculty of Medicine, Department of Obstetrics and Gynecology, Istanbul, Turkey

³Istanbul University, Istanbul Faculty of Medicine, Department of Pathology, Istanbul, Turkey

ORCID IDs of the authors: İ.D.K. 0000-0002-0179-2009; C.Y. 0000-0002-7183-1456; S.Ö. 0000-0002-1384-630X; E.Y. 0000-0002-3166-0648; S.T. 0000-0002-2658-6439; S.E.A. 0000-0002-9086-1060

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ABSTRACT

Objective: The aim of the study is to assess local immune activation through B lymphocytes density and the presence of germinal centers in the vestibular mucosa of patients with localized provoked vulvodynia (LPV).

Materials and Methods: Vestibulectomy specimens of 29 patients with LPV and colporrhaphy posterior specimens of 20 control women were included in the study. Immunohistochemical staining with CD20 antibodies for B lymphocytes were performed and subsequently, microscopical evaluation for B lymphocytes density and the presence of germinal centers was conducted.

Results: B lymphocyte densities were higher in the vestibular mucosa of patients with LPV compared to control women (87 vs 21, $p < 0.01$). The presence of germinal centers was only observed in the specimens of patients with LPV (0.31 vs 0, $p < 0.011$).

Conclusion: The presence of germinal centers with increased B lymphocyte density underlines a local immune activation in the vestibule of patients with LPV. An abnormal germinal center response can trigger the pathway to the development of autoimmunity through the emergence of an autoreactive B cell clone and autoantibodies. LPV can potentially emerge as an autoimmune disease of the vestibule. More studies are needed to understand the pathophysiology of LPV and its association with autoimmunity.

Keywords: Vulvar vestibulitis, B lymphocytes, germinal center

ÖZET

Amaç: Bu çalışmadaki amacımız, lokalize provoke vulvodinisini (LPV) olan hastaların vestibular mukozasında, B lenfosit yoğunluğu ve germinal merkez varlığına bakılarak lokal immün aktivasyon olup olmadığını araştırmaktır.

Gereç ve Yöntem: LPV olup vestibülektomi ameliyatı geçiren 29 hastanın kolporafi posterior ameliyatı olan 20 kontrol hastanın vulvar vestibüler doku kesitleri incelenmiştir. B lenfositini göstermek için kullanılan CD20 antikoru ile yapılan immünohistokimyasal boyamayı takiben mikroskop altında B lenfosit yoğunluğuna ve germinal merkez varlığına bakılmıştır.

Bulgular: Kontrol vakaların kesitleri ile kıyaslandığında, LPV olan hastaların vulvar vestibüler mukozasında B lenfosit yoğunluğu daha yüksek izlenmiştir (LPV hasta 87 vs kontrol hasta 21, $p < 0,01$). Germinal merkez varlığı sadece LPV olan hastaların vestibüler mukozada kesitlerinde izlenmiştir (LPV hasta 0,31 vs kontrol hasta 0, $p < 0,011$).

Sonuç: LPV hastalığında, vestibüler mukozada artmış B lenfosit yoğunluğu ve beraberinde izlenen germinal merkez varlığı, lokal bir immün aktivasyonun olduğunu göstermektedir. Anormal germinal merkez cevabının olması durumunda, otoreaktif B lenfositleri ve bunun sonucunda otoimmün hastalıklar gelişebilmektedir. LPV'nin patofizyolojisinin ve otoimmünite ile ilişkisinin ortaya konmasında daha fazla sayıda çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Vulvar vestibülit, B lenfosit, germinal merkez

Corresponding author/İletişim kurulacak yazar: irem.demiral@hotmail.fr

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INTRODUCTION

Vulvar vestibulitis syndrome (VVS), today described as localized provoked vulvodynia (LPV), is a disease showing three prominent characteristics: severe pain triggered by vestibular touch or vaginal entry, vestibular tenderness upon pressure and various degree of vestibular erythema (1, 2). With a prevalence up to 15% in premenopausal women, this disease seriously affects the sexuality and quality of life of patients because the pain can be triggered by any daily condition exercising pressure on the vestibule such as insertion of tampon, horse riding, wearing tight clothes but especially by vaginal penetration (3, 4).

The etiopathogenesis of LPV is not fully understood. Many factors such as genetics, hormonal factors and inflammation are found associated with the disease (5). Lymphocytic infiltration in the vestibular mucosa has been described (6, 7). However, the absence of classic active inflammation suggests the hypothesis of an excessive response and deregulated inflammation (4, 8, 9).

A local inflammation mediated by increased inflammatory cytokine TNF- α was demonstrated in the vestibule of patients with LPV (10). Another study analyzing different immune cells in the vestibule showed a local immune activation: the formation of germinal centers with increased B lymphocytes density was found specific to LPV (4). In this study, the researchers looked for B lymphocytes and germinal centers in the vestibular mucosa and to assess if the local immune activation was observed in the patients.

MATERIALS AND METHODS

Study subjects

The study included 29 patients with LPV diagnosis and 20 age, sex and race-matched women with no history of vulvodynia. The archival vestibulectomy specimens of patients with LPV treated surgically in the gynecology clinic of Istanbul University, Istanbul Faculty of Medicine between 2009-2016 were used.

The inclusion criteria for LPV were: vulvar pain upon attempted vaginal intercourse or tampon insertion lasting more than one year; tenderness upon touch with a cotton tip in the vestibular area limited between Hart's line and hymen; failure of less than six months medical treatment (1-3). Exclusion criteria were: (1) use of antibiotics or immunosuppressive agents for the last 30 days; (2) clinically present microbial infection; (3) any neurologic disease or other pain syndrome (1-3).

Vestibular pain scores were obtained using a visual analog score ranging from 0 for no pain to 100 for severe intolerable pain: patients with LPV were asked to report the intensity of the pain they perceived while vestibular

area was touched at 3, 5, 7 and 9 o'clock positions with a cotton tip. If the average of the 4 scores was higher than 36, surgical treatment (vestibulectomy) was offered to patients.

The control group involved age, sex and race-matched women who underwent posterior colporrhaphy for rectocele repair in the gynecology clinic of Istanbul University, Istanbul Faculty of Medicine between 2015-2016. The study was approved by the local ethical committee (Date: 12.09.2019, No: 80). All participants gave informed consent to participate in the study.

Immunohistochemistry evaluation

Tissues were fixed in 10% formalin for 24 hours then embedded in paraffin. Cut into 3-5 μ m sections, embedded paraffin tissue blocks were placed on microscope slides. The slides were incubated in 56°C for one night to dry out the sections. Sections were deparaffinized with xylene solution for 30 minutes, immersed in acetone for 5 minutes and in 96% ethanol for 15 minutes, then washed with distilled water. Afterwards, to block the endogenous peroxidase activity, sections were incubated in 3% hydrogen peroxide solution in methanol at room temperature for 10 minutes.

Once the sections were ready for immunohistochemical staining, incubation with antibodies against CD20 for B cells (clone 760-2531,1:1000; Ventana Medical Systems Roche Group, USA) were performed according to the manufacturers' instructions. The sections were subsequently incubated with anti-mouse antibody followed by exposure to streptavidin, horseradish peroxidase (HRP) conjugate. Then, 3-amino-9-ethylcarbazole (AEC) was added as substrate. Finally, all sections were counterstained with hematoxylin. All the procedures were performed in the immunohistochemistry laboratory of the Pathology Department of the Istanbul University School of Medicine.

Tissue analyses

The immunohistochemical scoring was done under light microscope at x40 magnification (Nikon Eclipse E800). Antigen stainings were assessed for density and localization in the vestibular mucosa. The evaluation was performed by taking the mean number of positive cells observed per field from 2-4 high-power fields (hpf). The number of germinal centers formed by B cells in the sections were also counted.

Statistical analysis

The SPSS 20 software (IBM Corp, Armonk, NY) was used for statistical analyses. In addition to descriptive analysis, the Mann Whitney U test was used for the comparison between patient and controls. A p value of <0.05 was considered statistically significant.

RESULTS

All subjects were Caucasian, from low-middle class and age, sex and race-matched. There were no statistical differences in the demographic findings of the two study groups.

CD20 is a marker of B lymphocyte appearing in the pre-B-cell stage and disappearing with differentiation to plasma cells (11). Anti-CD20 antibodies showed the presence of B lymphocytes. When compared with the sections of controls, the researchers observed that B lymphocytes are statistically significantly higher in patients with LPV (Table 1). B lymphocytes were observed mostly in the stromal tissue of the mucosa (Figure 1).

The presence of germinal centers formed by B lymphocytes was observed only in patients with LPV (Figure 2). The number of germinal centers varied between 1-2 in 8 sections (27% of patients with LPV) (Table 1).

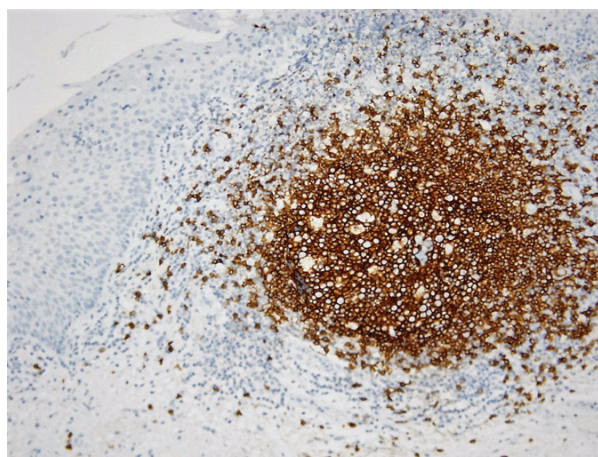


Figure 2: Germinal center in vulvar vestibular mucosa

A germinal center, formed by CD20+ immunostained B lymphocytes, in the sample of a patient with localized provoked vulvodynia. Histological sections were counterstained with hematoxylin and photomicrographed using x20 objective.

Table 1: The numbers of B lymphocytes and germinal centers in localized provoked vulvodynia

CellType	LPV (n=29) Mean (95%CI) ^a	Controls (n=20) Mean (95%CI) ^a	p value ^b
B lymphocytes	87 (58-129)	21 (12-32)	<0.001
Germinal centers	0.31 (0-2)	0 (0)	0.011

LPV: localized provoked vulvodynia, CI: confidence interval, ^a: Mean is obtained with cell counts per microscopy field, analyzed from 2-4 fields (x40 objective), ^b: Mann-Whitney U test

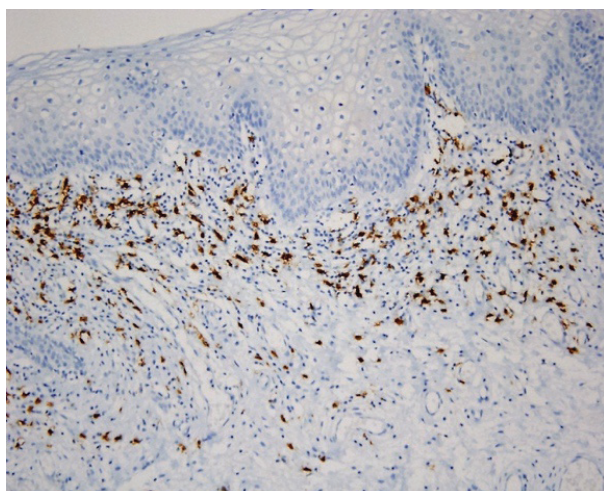


Figure 1: CD20(+) B lymphocytes in vulvar vestibular mucosa

Increased density of CD20+ B lymphocytes in a patient with localized provoked vulvodynia.

B lymphocytes were mostly observed mostly in the stromal tissue of the mucosa. Histological sections were counterstained with hematoxylin and photomicrographed using x20 objective.

DISCUSSION

This study is the first to assess local immune activation in the vestibulum of Turkish patients with LPV. The researchers observed significantly increased B lymphocytes in patients with LPV. The germinal centers were observed only in patients with LPV. In a previous study conducted on Finnish women, the comparison of vestibulectomy specimens between women with LPV and controls showed statistically significantly increased B lymphocytes in the vestibule of patients with LPV. The presence of germinal centers was also observed only in patients with LPV, like our results (4).

B lymphocytes are cells that recognize specific antigens via different immunoglobulins (Ig) anchored on the cell surface (12). Once a B lymphocyte recognizes an antigen, it differentiates into plasma cells that can secrete antibodies. Germinal centers are the microstructures where memory B cells and high-affinity antibodies secreting plasma cells are localized. It is important as it provides protection against reinfection (13). Recently, it was suggested that the germinal center response is the main pathway leading to autoantibodies found in autoimmune

diseases (14). Therefore, the germinal center response requires a very delicate balance and complex regulation to avoid the development of autoreactive B cell clones and subsequent autoimmunity (13).

The presence of germinal centers with increased B lymphocytes density in the vestibule of patients with LPV suggests a local immune activation. An 'unknown antigen' captured by B lymphocytes activated the humoral immunity and a germinal center response was given by the lymphoid tissue localized in the vestibule. Each time the vestibular mucosa is exposed to the 'unknown antigen', this cycle is triggered leading to a local inflammation in the vestibular mucosa, a feature demonstrated by previous studies (6). A constant exposure to the 'unknown antigen' contributes to the chronic persistence of the disease and the mainstay of the therapy will be avoiding the 'unknown antigen'.

A different perspective can be adopted for the etiopathogenesis of LPV: an abnormal germinal center response following a single exposure of an 'unknown antigen' can be the starting point in LPV. The differentiation of B lymphocytes in the germinal centers can be unsuccessful if autoreactive B cells are developed in the end of this mutation process (13). Normally, the self-tolerance mechanisms in the germinal centers prevent the autoantibody production from these autoreactive B cells (15). However, an abnormal germinal center response with deficient self-tolerance mechanisms can't block this process and autoimmunity will subsequently develop. The increased B lymphocyte density found in the vestibule can in fact point out these autoreactive B cells clones. As the production of autoantibodies from these autoreactive B cells are found associated with autoimmune disease, LPV can potentially emerge as an autoimmune disease of the vestibule (15).

The main strength of the study is the high number of women involved in both patient (n=29) and control (n=20) groups. All specimens in the patient groups were obtained by the same surgeon, all specimens in the study were pathologically examined by one physician, making any inter-observer variation unlikely. The main limitation of the study is that any other autoimmunity antibodies were not assessed in both groups to support a stronger association between LPV and autoimmunity.

CONCLUSION

Localized provoked vulvodynia remains a disease where more studies are needed to understand its pathophysiology. The 'agent' triggering the activation of VALT is not elucidated. The presence of germinal centers with increased B lymphocytes density underlines a local immune activation in the vestibule of patients with LPV. An abnormal germinal center response can trigger the pathway to the development of autoimmunity through

the emergence of autoreactive B cells clone and autoantibodies. The hypothesis of LPV being an autoimmune disease of the vestibule can be the subject of more studies that can reveal interesting findings. Hopefully, a better understanding of the disease will help in developing targeted therapeutics for LPV.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of the Istanbul University, Istanbul Faculty of Medicine (Date: 26.02.2016, No: 290).

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