Elevated tissue levels of tumor necrosis factor-α in vulvar vestibulitis syndrome

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Summary

The purpose of this study was to compare levels of inflammatory cytokines, namely TNF- α, IL-1β, and IL-1 receptor in women with vulvar vestibulitis syndrome (VVS) relative to levels in controls. The authors hypothesized that tissue concentrations of inflammatory cytokines would be elevated significantly in women with VVB compared to pain-free controls. The study population consisted of 15 women with strictly defined VVB in reproductive age and 13 age-matched women with no history of vulvodynia. For TNF-α, positive staining was observed in 40% of the samples from the study group and in 7.7% of the samples from the control group. The difference between the groups was statistically significant ($p < 0.05$). In conclusion, a limitation of the present study was the relatively small sample size. However, the authors' intention was simply to propose that the local inflammation may be mediated by cytokines as TNF-α may rather than trying to single out a pathogenesis of VVS. The authors' findings of elevated TNF-α may suggest new therapeutic alternatives for VVS, as inhibiting cytokine synthesis or antagonism of the cytokine receptor.

Key words: TNF- α; IL-1β; IL-1 receptor; Vulvar vestibulitis syndrome.

Introduction

Vulvar vestibulitis syndrome (VVS) was first described nearly a century ago [1] and is currently one of the most frequent causes of coital pain in women within premenopausal period [2]. However, gynecologists have only recently begun to pay more clinical attention to this intriguing syndrome.

Vulvar vestibulitis is a chronic clinical syndrome characterized by severe pain on vestibular touch or attempted vaginal entry, tenderness to a cotton-swab palpation of the vestibular area, and physical findings confined to vestibular erythema [3]. It has received increasing attention from a number of multi-disciplinary researchers, including gynecologists, dermatologists, urologists, psychiatrists, and psychologists. The syndrome is regarded as one of the subtypes of vulvar pain and dyspareunia [2, 4]. In addition, the suspected prevalence seems to be increasing, with reported rates of <15% [3, 5, 6].

The etiology VVS remains unknown and is probably multifactorial. However, the chronic inflammation might be related to a decreased ability to cease the inflammatory response due to prolonged induction of pro-inflammatory cytokines. Some researchers propose a neuropathic etiology for VVS, because thresholds to thermal and mechanical stimuli are lowered in VVS patients like in other neuropathic pain syndromes [7-9].

Recent studies propose that pro-inflammatory cytokines, like interleukin-1β (IL-1β) and tumor necrosis factor- α (TNF-α), may have a role in the pathogenesis of neuropathic pain [10, 11]. Furthermore, some studies suggest that these factors may aggravate inflammatory diseases [12-14].

The purpose of this study was to compare levels of inflammatory cytokines, namely TNF- α, IL-1β and IL-1 receptor in women with VVB relative to levels in controls. The authors hypothesized that tissue concentrations of inflammatory cytokines would be elevated significantly in women with VVB compared to pain-free controls.

Materials and Methods

Study population

The study population consisted of 15 women with strictly defined VVB in reproductive age and 13 age-matched women with no history of vulvodynia. The study was carried out in the gynecology clinic of Istanbul University School of Medicine.

Strict inclusion criteria were: (1) ≥ one year of vulvar burning with insertional dyspareunia or pain with tampon insertion; (2) tenderness to light touch, limited to the vulvar vestibule between Hart's line and the hymeneal tissue (cotton-tip applicator was used to record a visual analog scale of 1 to 10); (3) ≤ six-month medical therapy did not relieve the pain.

Exclusion criteria were: (1) usage of antibiotics or immunosuppressive medications in last 30 days; (2) a clinically apparent microbial infection; (3) any other neuropathology.

All perineoplasty procedures were performed by one of the authors (S.E.A) and vulvar vestibule was confirmed by a second independent examiner.

The controls were women that underwent rectocele repair (posterior colporrhaphy) in the last six months who were pain-free and had a normal neurologic exam.

This investigation was approved by the Ethics Committee of Istanbul University and written consent from the subjects were obtained prior to the initiation of the study.

Immunohistochemistry evaluation

Expression of TNF-α, IL-1β and IL-1 receptor in tissue samples was determined by immunohistochemistry. All tissue sam-

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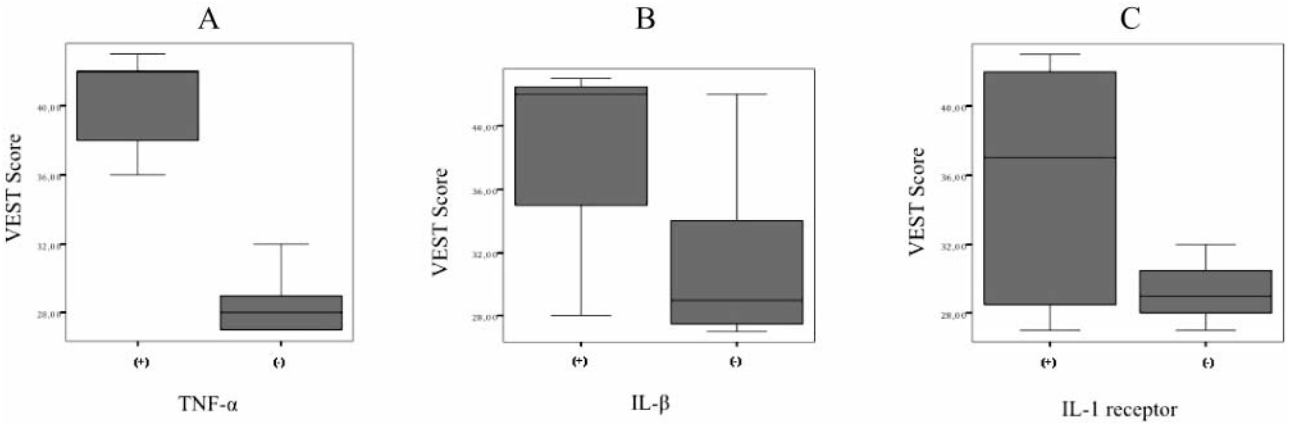

Figure 1. — VEST score for TNF-α, IL-1β and IL-1 receptor, respectively.

ples were collected from identical areas of vulva and vestibule in study group and controls. Tissues were embedded in paraffin, cut into sections three- to five-μ thick and incubated with the following antibodies: TNF-α antibody (dilution 1: 400), IL-1β antibody (dilution 1: 200), and IL-1 receptor antibody (dilution 1: 200). The tissue sections were then incubated with anti-mouse antibody followed by exposure to streptavidin, horseradish peroxidase (HRP) conjugate. Finally, 3-amino-9-ethylcarbazole (AEC) was added to serve as a substrate. Afterwards, glass sealed sections were viewed with a fluorescence microscope and photomicrographs were taken using a digital camera. The stained sections were observed by a single investigator who was blinded to the patient data. To avoid false-positive staining, fields were selected at a relative distance from tissue section margins.

Statistical analysis

Statistical analyses were performed using SPSS 15.0 software package. Descriptive and cross-tab analyses were conducted. A value of $p < 0.05$ was considered statistically significant.

Results

All subjects were Caucasian, from the metropolitan area of Istanbul, low-middle class, and were matched for age.

For TNF-α, positive staining was observed in 40% of the samples from the study group and in 7.7% of the samples from the control group. The difference between the groups was statistically significant ($p < 0.05$, Figure 1a).

For IL-1β, positive staining was observed in 40% of the samples from the study group, while in 69.2% of the samples from the control group. Although the positive staining ratio was higher in the control group, the difference was not statistically significant ($p = 0.12$, Figure 1b).

For IL-1 receptor, positive staining was observed in 53.3% of the samples from the study group, while in 84.6% of the samples from the control group. Although the positive staining ratio was higher in the controls, the difference was not statistically significant ($p = 0.08$, Figure 1c).

Discussion

The cause of VVS remains puzzling. The dominant theory is that VVS is a neuropathic disorder and involves abnormal pain perception due to sensitization of vestibular nerve fibers and the establishment of a sympathetically supported pain loop. Currently unidentified trigger events, probably some type of chronic inflammation, activate and cause extended involvement of the sympathetic, Type C nerve fibers. These fibers are responsible for transmitting noxious chemical or thermal stimuli to the brain. This process leads to an abnormal response of the wide dynamic range neurons in the brain. Hence, mild stimuli are perceived as pain by the patient. Consequently, there is first localized pain of VVS which progresses to the chronic, generalized vulvar pain of dysesthetic vulvodynia [15].

Neuropathic pain happens due to damage or inflammation of the nervous system [16, 17]. Evidence suggests that proinflammatory cytokines, like IL-1β and TNF-α, contribute to the pathogenesis of neuropathic pain [10, 11]. These cytokines are upregulated in the injured peripheral nerves, stimulating chronic neuroinflammation and leading to neuropathic pain [18, 19].

Blocking TNF-α pharmacologically and genetically has prevented neuropathic pain in various studies involving neuropathic pain models [20, 21]. Additionally, some studies presented that administration of TNF-α into the peripheral pain transmission pathways produces a pain response like neuropathic pain [22]. Observed elevated levels of TNF-α in tissue samples of patients with VVS in the present study are in line with the aforementioned association between VVS and neuropathic pain.

IL-1β is a well-characterized cytokine, which is produced by macrophages, and Schwann cells, activating other inflammatory cells [23]. Accumulating evidence suggests that IL-1β may play a vital role in the generation of mechanical hyperalgesia [24]. Considering the well-localized hyperalgesia of the vestibule, the non-significant lower levels of

IL-1β in tissue samples of patients with VVS in comparison to controls in the present study is unanticipated. The low concentration of IL-1β might suggest that cytokines may not be the common pathway to hyperalgesia. Cytokines might increase regionally as a response to trauma and/or inflammation. Of course, the present unanticipated findings could be based upon artefactual differences in cytokine levels at different sampling sites as well.

In studies with animal models, inflammatory hyperalgesia was prevented by administration of endogenous IL-1 receptor antagonist (IL-1ra) [25, 26]. These studies suggested that neutralizing antibodies to IL-1 receptors reduced pain-associated behavior in mice with experimental neuropathy. Interestingly, reduced induction of IL-1ra was observed in the blood samples of patients with VVS compared to controls [27]. Similarly, IL-1 receptor levels were lower in tissue samples of patients with VVS in the present study.

In conclusion, a limitation of the present study was the relatively small sample size. Hence, these findings cannot be generalized to the wider patient group. However, the present authors' intention was simply to propose that the local inflammation may be mediated by cytokines like TNF-α may rather than trying to single out a pathogenesis of VVS. The findings of elevated TNF-α may suggest new therapeutic alternatives for VVS like inhibiting cytokine synthesis or antagonism of the cytokine receptor.

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