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Sensory Neuropeptides, Serotonin Innervations and Personality in Women with Vulvodynia

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Abstract

Objective: The pathophysiology of vulvadynia is still unclear. We evaluated the general innervations of the nerves, the sensory neuropeptides in the vestibular area in eighteen women with vulvadynia, aged 20-38. The same individual acted as her own control and biopsies were taken from affected and none affected areas of posterior wall of the vulval vestibule. Quantitative immunohistochemistry was performed, using antisera to the general neuronal marker protein gene product (PGP) 9.5 and to the sensory neuropeptides substance P and calcitonin gene-related peptide (CGRP). Pain and stress assessments were made.

Results: The number of PGP 9.5 immunoreactive in the affected areas showed an increase in number (p=0.06) compared to control sites (p=0.40), but the result did not reached to significance. High scores for pain sensation, signs of burnout, emotional and physical symptoms of stress and anxiety were indicated, regardless of time in all women.

Conclusions: An increase in PGP 9.5 immunoreactive nerve fibers may be either secondary to nerve sprouting, or may represent neural hyperplasia, which could be applied as an objective diagnostic finding in vulvadynia. Further studies needed for the neuromediator's roll and inter individual factors for the diagnosis. Vulvodynia seem lead to chronic pain, hence having negative impact on the couple's relationship and the quality of life of women in several ways.

Keywords: Protein gene product 9·5; Vestibular innervations; Vulvodynia; Dyspareunia; Neuropathic pain

Introduction

Vulvodynia is a heterogeneous, multisystemic and multi-factorial disease in women of fertile age. It is characterized by the sudden onset of a painful burning sensation, hyperalgesia, mechanical allodynia localized to the region of the vulval vestibulus [1-4].

These patients have not diagnosable dermatologic or medical condition that explains the cutaneous symptoms. The skin regions look normal but have a pain localized surrounding area of the Bartholin gland. Sensory cutaneous nerve fibers as well as epidermal and dermal immune cells are capable of producing neuromediators in the skin, which can activate specific receptors on target cells and thus modulate physiological and pathophysiological effects. In addition these neuromediators have been found to activate a number of cutaneous cells via a signaling cascade.

Substance P and CGRP are proinflammatory peptides which are present in C-fibers with a number of effects, for example, by binding to neurokinin 1 receptor (NK1R) substance P has an important role in pain and hyperalgesia [5]. Neurokinin 1 receptor which can be found in both the central and peripheral nervous system, has also been associated with the transmission of stress signals and pain.

Like other sensory neuropeptides, the substance P can be released from the peripheral terminals of sensory nerve fibers in the skin, muscle, joints and involves in the integration of pain, stress, and anxiety. Furthermore, it is suggested that, the substance P play a role in nociception by the reduction of response thresholds [6].

Serotonin (5-hydroxytryptamin; 5-HT) acting via 7 families of receptors and is essential for numerous basic cellular functions and has a widespread distribution in the CNS and periphery. In addition serotonin has an enormous ability to interact with other neural networks; neurotransmitters are involved in the regulation of a number of physiological functions and are an important mediator in stress and anxiety [7].

An increased innervations and/or sensitization of thermo receptors and nociceptors have been seen in vestibular mucosa in this group, whom has significantly lower vestibular pain thresholds in this area. Given that the pain might be triggered by peripheral nerve injury or by the release of neuronal mediators, we wanted to evaluate the general innervations using PGP 9.5 and the sensory neuropeptides substance P with its receptor neurokinin 1 (NK1), neurochemically characterize the superficial nerves in women with vulvadynia and areas without pain sensation as control sites [8,9]. To our knowledge, no other group had same individual as her own control.

Method and Participants

Participants were recruited by the same gynecologist working at outpatient ward at Karolinska University Hospital. Eighteen women between 18-40 years diagnosed with vulvodynia who met eligibility criteria, included in the study and classed as their own controls. Women with sexual dysfunction present before the onset of vulvodynia and fibromyalgia diagnosis, and ongoing treatments for these conditions were excluded. Complete medical history, including previous and or present diseases; previous and or ongoing treatments were obtained. Physical examination for the assessment of gynecological health and to exclude vaginismus, other vulvar/pelvic pain and/or pain during intercourse and infections were done in a standardized manner. The vaginal cultures was taken to evaluate if bacterial vaginosis, trichomonas or candida presence. If Pap smear were taken more than a year ago, new test were carried on.

The cotton-swab test was performed by same gynecologist, the palpation of six vestibular sites in terms of clock positions: (12,12-3,3-6,6-9,9-12 O'clock) around the hymeneal ring. The visual analogue score (VAS) of pain experienced upon attempt of sexual contact, and during examination was used as a marker of severity, were evaluated on a 0 (absence of pain) to 10 (the worst pain ever experienced) manner [10,11]. Participants were asked to fill in questionnaire consists of stress at private life, at work, negative life events, Quality of Life (QoL) and the questionnaire, Karolinska Scales of Personality (KSP) was used and the purpose of the test was to identify personality traits related to psychological vulnerability.

Skin biopsies and the control biopsies were taken from same participant, where pain could and could not objectively elicit in the examined vestibular areas.

The study was approved by the ethical committee of the Karolinska Institute. Written informed consent was obtained from each participant.

Pain

Pain assessment was conducted using these measures: vulvodynia has been described according to Friedrich criteria; symptoms and signs as 1) severe pain on vestibular touch or attempted vaginal entry; 2) tenderness when pressure is localized within the vestibule; and 3) physical findings confined to vestibular erythema of various degrees [1].

Sampling and preparation of the tissue

A general neuronal maker was used for evaluation of sensory cutaneous nerve fibers which detects in turn cutaneous nerves and a protein gene product 9.5 (PGP 9.5) as general neuronal marker, and GAP-43 (growth-associated protein-43) who plays a key role in directing the growth of axons and modulating the formation of new neural connections [12,13].

The samples were obtained after anesthesia using xylocaine-adrenaline infiltration. A 3 mm punch biopsy was then taken in the surrounding area of the Bartholin gland, were the pain were localized. Beside this area the participants had no pain and/or visible erythema either in the vulva or in the vagina. A control biopsy was taken at the corresponding contra lateral side, where no pain sensations elicitated.

The biopsies were immediately immersed in Lana's fix, containing Paraformaldehyde-picric acid, 2h in 4°C and then rinsed in 10% sucrose in Sörensen's phosphate buffert solution, PBS overnight. The samples were then frozen at -70°C and embedded with OCT (Optimal Cutting Temperature Compound). Cryostat sections 14 µm thick, cut in a right angle to the surface, were applied in three paired groups to glass slides resulting in six sections from each biopsy and refrozen at -70°C.

Skin biopsies

Cryostat-cut Sections (14 µm) of the skin biopsies were prepared on a Microm Dittes cryostate, and the cryosections were then mounted on Super Frost Plus glass slides (Menzel-Gläser, Freiburg, Germany) and stored at -70°C until being used for immunohistochemistry.

Immunohistochemistry

After drying the samples in the room temperature, they were then rinsed with PBS in for five minutes and afterwards rinsed in 0.3% H_2O_2 in PBS for 15 min in darkness. Slides were then thoroughly rinsed at least four times in PBS and dried before the individual section pairs were separated by a PAP-pen (liquid repellent slide marker pen). All the sections were covered by a 10% goat serum blocker (Vector Laboratories, USA) in 1% BSA (Bovine Serum Albumin, Sigma, Germany)/PBS for 40 min at room temperature.

The primary antibodies produced in a biotinylated-streptavidine technique were used to detect all antibody-labeled molecules. The slides were incubated overnight (4°C) in a humid chamber with primary polyclonal antibodies (all diluted 1:20,000) against human PGP 9.5 (polyclonal, rabbit, Ultraclone, Cam, United Kingdom); substance P, CGRP, (Bachem, St Helens, United Kingdom); GAP-43 (diluted 1:3000) (Chemicon, Temecula, Calif); and a rabbit polyclonal rabbit antibodies against 5-HT (DiaSorin, Stillwater, MN, USA).

On the following day the sections were rinsed in phosphate-buffered saline and incubated with a biotinylated goat antirabbit secondary antibody or, in case of the monoclonal antibody, with a biotinylated horse antimouse secondary antibody (both diluted 1:200) (Vector, Burlingame, Calif) for 40 minutes. The primary antibodies were visualized by incubating the sections with the fluorochrome Cy2 (dilution 1:2000) (Amersham Pharmacia Biotech, Uppsala, Sweden).

Microscopy

The labelled sections were examined under a Nikon epifluorescence microscope (Eclipse E800, Yokohama, Japan). Cy2 and FITC fluorescence were visualized following excitation at 465-495 nm and Texas Red fluorescence following excitation at 540-580.

Digital photographs (Nikon DXM 1200) of the stained slides were then coded and examined blindly by one of the authors (SLR).

In the biopsies sections at three levels encompassing the whole biopsy were examined, and the mean values of the studied various markers were determined.

Psychological distress

For assessment of personality, the self-reported personality inventory, the Karolinska Scales of Personality (KSP), The KSP is a questionnaire consisting of 135 items with four-point response scales. The items are grouped to form 15 scales; Impulsiveness, Monotony, Avoidance, Detachment, Socialization, Social Desirability, Psychic Anxiety, Somatic Anxiety, Muscular Tension, Psychasthenia,

Inhibition of Aggression, Verbal Aggression, Indirect Aggression, Irritability, Suspicion, and Guilt. Scales included in the inventory have been classified on the basis of factor analyses into four groups: (i) Impulsivity, sensation seeking and withdrawal scales; (ii) Psychopathy vs. conformity scales; (iii) Anxiety-related scales; and (iv) Aggressivity-related Aggression scales and Hostility scales.

Statistical analysis

The mean densities of fibers staining positively for the various neuropeptides and receptors were determined for each biopsy. Thereafter the overall mean ± standard deviation and median values for the patients were compared to the corresponding control values. Comparisons between affected area and non-affected were performed with the t-test for independent samples. The significance level was set at $p<0.05$.

Results

The clinical characteristics of study population are shown in Table 1. None of the women had candida vaginosis, or tricomona infections. The duration of vulvodynia differed between more than two years and 19 years. Seven of the women had, had also other diagnosis, of which, six was psychiatric diagnosis, but not ongoing treatment. Eleven women classified as otherwise healthy. None of the biopsies showed presence of acute or chronic inflammation, since it is considered as a normal finding in vestibular tissue and does not serve as a histological marker for vulvodynia. The PGP and CGRP showed tendency to significance in the pain area ($p=0.06$) compared to control sites ($p=0.40$).

	Women with VVS (n=18)
Mean age (years)	27.0+5.7
Mean education (years)	16.25
Body Mass Index (BMI), kg/m^2	22.5+1.8
Marital status	
No partner	1
Regular partner/cohabiting	17.0 (94.4 %)
Pregnancies	2.0 (11.1%)
Childbirth	2
Nulliparous	16 (89%)
Other diseases	
Medical conditions	1
Psychiatric conditions	6 (33%)
Mean age at first intercourse (years)	16
Mean time since onset of pain (years)	20

Table 1: Anthropometric and clinical variables in women with Vulva vestibular syndrome

The pain sensation showed the worst pain ever experienced during the examination and same high score was mentioned at attempt to sexual penetration for all. Seventy-two percent of the women had interrupted act of sexual intercourse due to unbearable pain and forty percent reported unsatisfactory sex due to this pain. All women (17/18) who had a partner were concerned over the relationship. The stress questionnaire KSP showed more signs of burnout and emotional and physical symptoms of stress regardless of free time or at work in all women.

Discussion

The pathophysiology of vulvodynia is still unclear. The main aim of this study was to evaluate the general innervations, using PGP 9.5 and the sensory neuropeptides substance P with its receptor neurokinin 1 (NK1) and neurochemically characterize the superficial nerves in women with vulvadynia, by taking biopsies from, same woman, affected and none affected sections of this area. Moreover if pain, its effects on intimate life of the participants.

There was an increase of PGP 9.5 immunoreactive nerve fibers in the affected sites of the vestibule, showing tendency to significance, without reaching it as it was shown earlier, still this founding reinforces the previously shown results [14].

Vestibule vulvae are covered by a sensitive mucosa of endodermal origin with increased intraepithelial innervated sections and the cutaneous sensory disorder might include these areas [14,15]. During sexual intercourse the vestibule is exposed to mechanical attrition, which can give up to intensive pain experience in women with vulvodynia. Although six women, one of them single, with vulvodynia reported not having penetrating sex, despite attempts, more than a year than the rest of the population, there were no significant differences in self-reported stress ratings and worse quality of life between them and the other twelve in the cohort. Can pain sensation, experience might be due to expected fear or overestimation of the pain? As several recent studied have indicated, our results was also correlated with high levels of stress in women with vulvodynia [16,17].

Biomedical factors are recognized to contribute to the pathophysiology of vestibulodynia but current conceptualizations of pain and sexual functioning suggest a multifactorial and biopsychosocial models [18-21]. The concept of "neuropathic pain" is gaining increasing attention in the scientific literature [20]. A significantly lower vestibular pain threshold was found in women with vulvodynia compared to control subjects and the pain was elicited by pressure to the vulvar vestibule or attempted vaginal penetration [2]. An impaired sexual functioning, increase psychological, marital distress and diminished quality of life due to the chronic recurrent pain sensations might be the result as shown by including this study group [21].

The popular notion that, psychological and sexual impairment is necessarily a direct consequence of pain intensity in women with vulvodynia was not supported by a recent study but all women in our study indicated by VAS-scale, pain as the main cause [18]. The psychiatric diagnosis was obtained several years after the problem with vulvodynia occurred, and whether these can maintain pain sensation or not unclear. One can presume that the chronic pain combined with both psychosocial and lifestyle factors might exacerbate the notion of the pain as Mundal et al. work indicated that chronic widespread pain in healthy population influence the risk of pain onset [18]. Psychological impairment, which is common in many patients, rather seems to be the consequence of the chronic disease than a primary condition.

Pain precipitated in the absence of nociceptor stimuli might be triggered by previous peripheral nerve injury, or by the release of neuronal mediators, which set off inappropriate impulses in nonmyelinated pain fibers sensitizing the dorsal horn neurons. Damage to the sympathetic nerves with increased pain sensitivity is the likely explanation for the burning sensation [19].

Vestibular glands of the vulva contain neuro-endocrine cells which are up regulated with inflammation and increases in the number of cells expressing serotonin [22].

The substance P showed tendency to significance on the affected areas compared to control sites in this study. What does it means that the same woman has different innervations, in the same area of vestibule? The increase of PGP immunoreactive nerve fibers in this group of patients, may be either secondary to nerve sprouting, or may represent neural hyperplasia. Even not significance, the increase of PGP immunoreactive nerve fibers may be applied as an objective diagnostic finding in vulvodynia as it was found by Tympanidis et al. [16].

Calcitonin gene-related peptide (CGRP) is a classic molecular marker of peptidergic primary somatosensory neurons. It is still unknown whether these neurons are required to sense pain or other sensory stimuli but increased number of PGP immunoreactive nerve fibers might indicate that these neurons have a function in pain sensation [23-26].

The diagnosis requires at least six months duration of provoked pain. Even though, the fact that the material included small number of participants, the duration of vulvodynia differed but the magnitude of the pain experience was very similar within the group.

The impact, the vulvodynia, has on the affected person seem to increase over time, with complex adaptation of, and changes in her psychological well-being, coping style, as well as in couple and family dynamics. As previously shown, a high percentage of the women in this study had interrupted act of sexual intercourse due to unbearable pain and unsatisfactory sex and in addition the women expressed fear for the relations existence [27,28].

Even though five of 18 women had psychiatric conditions, there is no compelling evidence that vulvodynia per se is associated with any particular psychological or behavioral characteristics other than the sort of difficulties in sexual functioning which might be expected with chronic vulval pain. However there is evidence for high levels of psychological distress in some samples of women with vulvodynia being seen in secondary care [23-25].

An accurate clinical diagnosis of different biological, psychosexual and relational factors that may act as either vulnerability sexual traits, precipitating and/or reactive factors should be carried out to establish the treatment (s) that may better address the etiologic complexity of vulvodynia. Future research in the area is needed [29].

Several limitations of this design should be considered. The substance P showed tendency to significance on the affected areas compared to control sites and it is very possibly that with a bigger cohort, the significance could be achieved. A healthy control group could help the significance of the PGP immunoreactive nerve fibers involment clearly. The data also only included patient ratings of sexual functioning. Inclusion of partner rating would enrich the clinical picture beyond the patient perspective which may be negatively biased secondary to the specific factors like body dissatisfaction, interpersonal traits. For the same reasons a healthy control group was not included. Further research, overcoming the limitations of the present study, is fully warranted.

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