Neuronal Nitric Oxide Synthase Expression in the Anterior Vaginal Wall of Patients with Stress Urinary Incontinence

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Purpose: Stress urinary incontinence (SUI) and pelvic organ prolapse (POP) are common medical problems, particularly among older women. In this study, we aim to explore the relationship between the neurotransmitter nNOS in the vaginal epithelium, and the occurrence of SUI and changes of nNOS levels according to menopausal status.

Matherials and Methods: Fourty women were enrolled. The patients were divided into four groups according to menstruaiton status and SUI. The vagina specimens were taken during transobturator tape application. The specimens were examined pathologically in terms of n-NOS expression. nNOS expression was compared between SUI and control groups. The results were evaluated statistically.

Result: Epithelial total nNOS score in group 1 and group 3 were 2.4 ± 0.5 and 1.4 ± 0.5 respectively ($P = .003$). Stromal total nNOS score was found 2.2 ± 0.4 in group 1 and 1.3 ± 0.5 in group 3 ($P = .001$). Epithelial total nNOS score in group 2 and group 4 were 4.4 ± 0.5 and 3.5 ± 0.5 respectively ($P = .003$). Stromal total nNOS score was found 4.4 ± 0.5 in group 2 and 3.6 ± 0.5 in group 4 ($P = .006$).

Conclusion: Our results show that expression of nNOs in the anterior vaginal epithelium decreased significantly in the SUI group. Altough our findings indicate important results, well designed further studies are needed to comprehend the role of NOS pathways better in SUI pathophysiology.

Key words: human vaginal tissue; nitric oxide; pathophysiology; pelvic floor disorders; stress urinary incontinence

INTRODUCTION

Stress urinary incontinence (SUI) and pelvic organ prolapse (POP) are common medical problems, particularly among older women. They can have a significantly negative impact on the quality of life, and yet less than half of women with urinary incontinence seek medical attention. These two diseases have an etiologically close relationship.[1]Pelvic floor tissues, including vaginal wall tissues, are rich in nerve fibers and functionally protect the normal position of pelvic organs.[2] The effects of pelvic support weakness may induce the development of SUI.[3] Changes in some neuropeptides in the pelvic floor tissue have been observed in SUI and POP patients.[4] Vasoactive intestinal peptide (VIP) is one of the neuropeptides widely distributed in the vaginal wall that serves as a local neurotransmitter or neuromodulator. However, the underlying neuropathophysiology of SUI is unclear. Nitric oxide (NO) is an anorganic free-radical gas that stimulates soluble guanylyl cyclase activity and creates smooth muscle relaxation.[5] There are three NOS isoforms:, neuronal (nNOS), endothelial (eNOS) and inducible (iNOS). Neuronal NOS (nNOS) is present in neuronal tissues; neuronal excitation increases Ca+2 concentration within nerves, which leads to synthesis of NO from L-arginine. Elucidation of the physiologic role of NO and nitric oxide synthase (NOS) is not only of research interest but may also provide a basis for therapeutic interventions in patients with lower urinary tract dysfunction. It is also important as a mediator in the female and male reproductive tracts.(6-8) In this study, we aim to explore the relationship between the neurotransmitter, nNOS in the vaginal epithelium, and the occurrence of SUI and changes of nNOS levels according to menopausal status.

PATIENTS AND METHODS

Study population

Institutional review board approval was obtained and all participants signed an informed consent before being enrolled in the study. Patients who attended our clinic with the complaints of incontinence and were detected to have SUI were included in the study. To evaluate incontinence, detailed incontinence history, age, body mass index (BMI), number of deliveries and previous surgical history were recorded. On physical examination, we accessed the women for SUI at gynecologic position, and we performed cough stress test.

Fourty women were enrolled. The patients were categorized into four groups according to menstruaiton status and SUI. Premenopausal SUI formed group 1, premenopausal controls formed group 2 and postmenopausal SUI formed group 3, postmenopausal controls formed group 4. Each group had 10 cases. The vagina specimens were taken during transobturator tape application. The specimens were examined pathologically in terms of n-NOS expression. The results were evaluated statistically. Control groups were choosen among healty women who had surgery for benign reasons such as

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Table 1. Characteristics of patients and comparison of nNOS expression among the four groups.

	Group1	Group2	Group3	Group4	*p*
Age	47.1 ± 5.1	46.5 ± 4.1	50.2 ± 2.7	49.6 ± 2.9	.126
Paritiy	3.2 ± 1.1	2.8 ± 1.4	3.2 ± 1.3	2.9 ± 1	.781
BMI (kg/m^2)	30.5 ± 3.3	27.8 ± 2.9	29.6 ± 1.3	28.3 ± 2.5	.126
nNOS epithelial	2.4 ± 0.5	4.4 ± 0.5	1.4 ± 0.5	3.5 ± 0.5	.001
nNOS stromal	2.2 ± 0.4	4.4 ± 0.5	1.3 ± 0.5	3.6 ± 0.5	.001
nNOS epithelial	2.4 ± 0.5		1.4 ± 0.5		.003
nNOS stromal	2.2 ± 0.4		1.3 ± 0.5		.001
nNOS epithelial		4.4 ± 0.5		3.5 ± 0.5	.003
nNOS stromal		4.4 ± 0.5		3.6 ± 0.5	.006

ureterorenoscopy or urethral caruncle.
Excluded from the study were women who had previous endometriosis, adenomyosis, uterine fibroids, connective tissue disorders, pelvic inflammatory conditions, or pelvic surgery. None of the patients took hormonal drugs during the 3 months before surgery. And none of the patients had POP.

Immunohistochemistry

The tissue sections were fixed in 10% buffered formalin for about 24 h, embedded in paraffin, and 5 µm sections were then deparaffinized. Then the sections were immersed in antigen retrival solution (Biogenex) and treated in microwave oven for 10 minutes. After cooling, the sections were washed with Phosphate Buffered Solution (PBS). The incubation with the primary antibody was done in a solution of 0.8% BSA and 20 mM NaN3 in PBS containing the nNOS–specific mouse antibody (1:100 dilution) for 1 h at room temperature. After rinsing with PBS the sections were incubated with the secondary biotinylated goat antimouse antibody for 30 min at room temperature. Then, an alkalene phosphatase complex was utilized as a detection system (1:200 dilution) for another 1 hr. Finaly, the staining was developed for 15 min with fast red solution and counterstained with Mayer's hematoxylin. Negative controls sections were incubated in the absence of the primary antibody.
The nNOS immunoreactivity of the specimens were rated according to a score that was calculated using intensity and area scores. The intensity of staining was on the following scale: 0, no staining of nNOS in the vagina; 1+, mild staining; 2+, moderate staining and 3+, marked staining. The area of staining was evaluated as follows: 0, no staining of in the vagina in any microscopic field; 1+, 0-25% of the vagina stains positive; 2+, 25-50% staining positive; 3+, 50-75% staining positive; 4+, 75-100% staining positive.

Statictical Analysis

All statistical analysis was performed by using SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA). Epithelial and stromal scores of nNOS were compared in four groups using Kruskal-Wallis H test and Mann-Whitney U test. In all comparisons of values, p-values of less than 0.05 were considered to be statistically significant.

RESULTS

There were no significant differences in age, BMI (calculated as weight in kilograms divided by height in meters squared), or parity among the four groups. Characteristics features of the patients are shown in **Table 1**.
nNOS immunostainings were shown in the anterior vaginal wall in all groups. nNOS immunostainings at different density were observed , as demonstrated by a mild, moderate, or marked staining accordingly. In women with SUI, only scattered nNOS with weak staining was observed. (**Figures 1, 2 and 3**). However, in postmenopausal women without SUI, the intensity and distribution of nNOS decreased and showed dispersed and moderate staining (**Figures 4 and 5**). Marked epithelial and stromal staining with nNOS was observed in a premenopausal patient without SUI (**Figures 6,7**). Comparison of nNOS expression is shown in Table 1. As shown in Table 1, the group with the most decreased

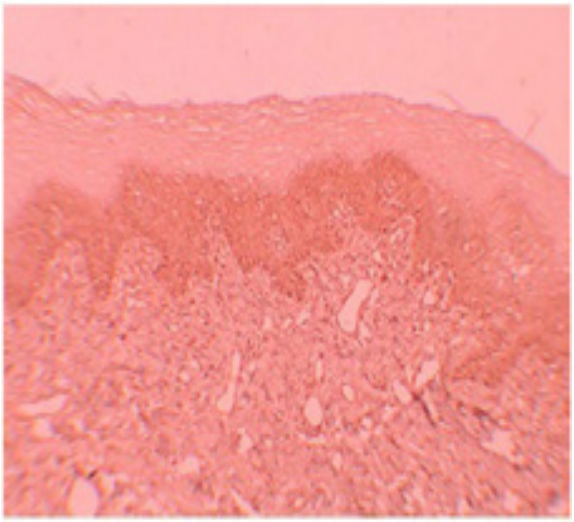

Figure 1. Mild epithelial staining (1+) with nNOS in a patient premenopausal with sui Immunostaining x100

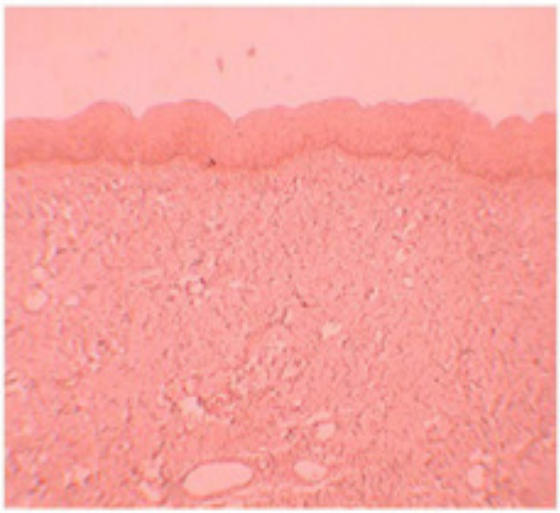

Figure 2. Mild epithelial staining (1+) with nNOS in a patient postmenopausal with sui Immunostaining x40

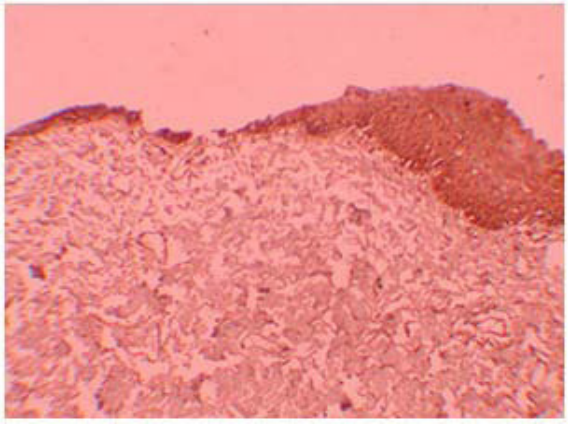

Figure 3. Mild stromal staining (1+) with nNOS in a patient postmenopausal with sui Immunostaining x100

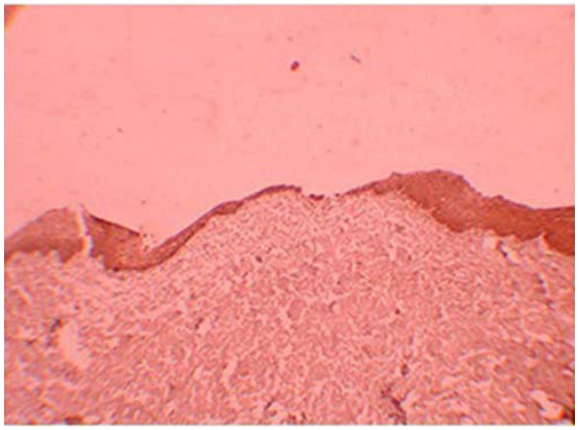

Figure 4. Moderate epithelial staining (2+) with nNOS in a patient postmenopausal without sui Immunostaining x100

expression of nNOS is Group 3.

Epithelial total nNOS scores in group 1 and group 3 were 2.4 ± 0.5and 1.4 ± 0.5, respectively with statistically significant differences between the two groups (*P* = .003). Stromal total nNOS score was found to be 2.2 ± 0.4 in group 1 and 1.3 ± 0.5 in group 3. There were statistically significant differences between the two groups (*P* = .001) (**Table 1**) (**Figures 1,5**)

Epithelial total nNOS score in group 2 and group 4 were 4.4 ± 0.5and 3.5 ± 0.5, respectively with statistically significant differences between the two groups (*P* = .003). Stromal total nNOS score was found to be 4.4 ± 0.5 in group 2 and 3.6 ± 0.5 in group 4. There were statistically significant differences between the two groups (*P* = .006) (**Table 1**) (**Figures 3,4**).

DISCUSSION

Pelvic floor disorders (PFD),including POP and SUI, are major health problems in women. The causes of PFD are multifactorial, including defect in the pelvic floor musculature and connective tissue weaknesses.[9] Another study has suggested that damage to the innervation of the pelvic floor can be an important factor in the etiology of SUI.[10] Neuropeptides, which are considered a marker of nerve damage, are used in the study of pelvic floor dysfunction. A neuropeptide is a peptide released by different tissues, that acts as a neural messenger. Neuropeptides act as neurohormones, neurotransmitters, or neural modulators. Neuropeptides are widely distributed in the central and peripheral nervous system and in many areas of the human body, including the gut, pancreas, heart, lung, and genital tract.[11] Neuropeptides are well-represented in the innervation of the human female reproductive tract. Neurons that contain vasoactive intestinal polypeptide (VIP) are abundant in the vagina, where they innervate blood vessels and smooth muscle in the vaginal wall, and form a plexus beneath the epithelial layer.[12] Neuropeptide Y (NPY) is also abundant in neurons in the human female genital tract, and is contained in nerves innervating blood vessels, as well as in nerves that form a subepithelial plexus.[13]

Previous studies have shown that VIP levels were significantly decreased in the anterior vaginal wall in premenopausal and postmenopausal SUI or POP patients. Hu et al. found that the expression of VIP in the vaginal epithelium significantly decreased in POP patients.[14] In

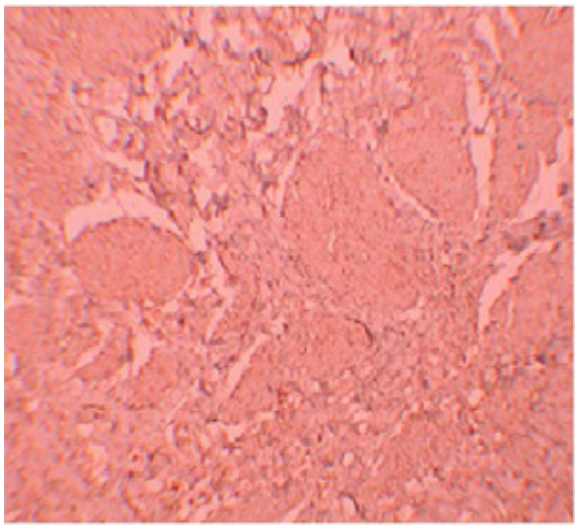

Figure 5. Moderate stromal staining (2+) with nNOS in a patient postmenopausal without sui Immunostaining x100

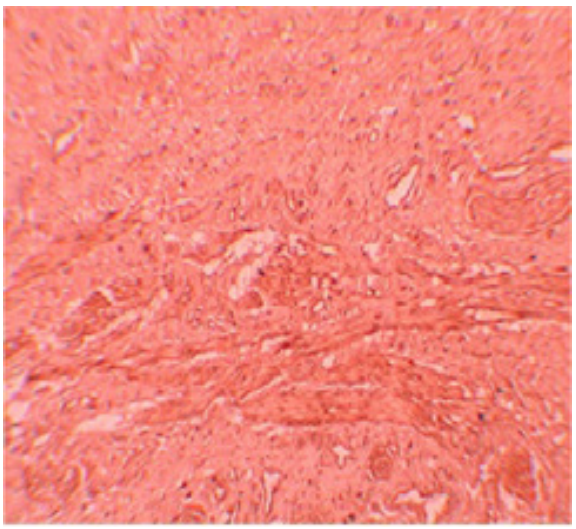

Figure 6. Marked epithelial staining (3+) with nNOS in a patient premenopausal without sui Immunostaining x100

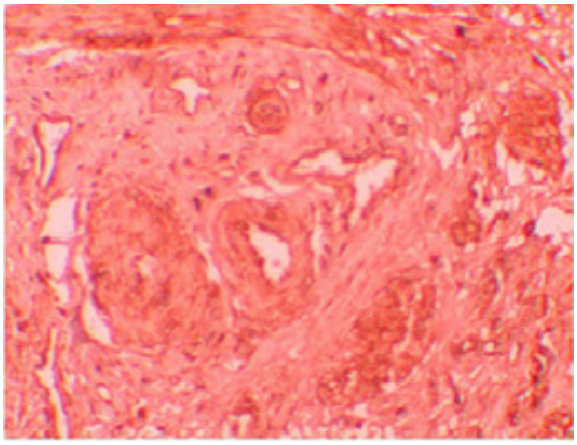

Figure 7. Marked stromal staining (3+) with nNOS in a patient premenopausal without sui Immunostaining x100

another study, Falconer et al. showed that women with SUI have a significantly lower total innervation of the paraurethral vaginal epithelium than controls without incontinence.[15] Alm et al.[16] reported the existence of vasoactive intestinal peptide (VIP) containing nerves in the vaginal wall. VIP-containing nerves have been described throughout the human female genital tract, and are most abundant in the vagina, cervix, and clitoris.[2] From studies on laboratory animals it has been suggested that NO may also function as a neurotransmitter in male and female genital organs, including the uterus and vagina.[17-20] and can mediate neurogenic vasodilatation. Hoyle et al. reported that nerves that utilise nitric oxide, NPY, VIP, or CGRP as a neurotransmitter may play a role in controlling blood flow and capillary permeability in the human vagina.[21]

Nerve and supportive tissue injury caused by prior pregnancy and delivery increasingly deteriorates as the patients ages.[22] As a component of the "hammock" according to the theory presented by Delancey[23], the anterior vaginal wall with decreased neurotransmitter content might alter the tension of the hammock, which contributes to the pathogenesis of SUI.

In light of previous studies we hypothesized that NOS secreted by the neuropeptidergic fiber terminals take part in the pathogenesis of SUI. In the present study we investigated nNOS expression in the anterior vaginal wall because the anterior vaginal wall plays a more important role than the posterior vaginal wall in the etiology of SUI[11]. Our results confirmed that nNOS levels decreased significantly in the anterior vaginal wall in SUI patients, supporting the possible role of nNOS in the pathophysiologic process of SUI.

Another result of this study is that, nNOS expressions are related to menopausal status. Compared to premanopausal control patients with postmenopausal control patients, nNOS levels decreased in postmenopausal period. This result shows clearly nNOS expressions is decrease in postmenopausal period.

The precise mechanism for the decrease of nNOS remains unclear. Lower nNOS in patients with SUI may either be related to the lower neuronal production of NOS or to age-induced nerve degeneration. Another possible explanation is that nNOS alterations affect the pathophysiological process via the local blood supply and nutrition state.

In summary, the present study constituted a specific assessment of the relationship between nNOS expression and the development of SUI. The present data could not determine whether differences in nNOS innervation are the cause of SUI or whether they result from the pathological changes caused by SUI.

CONCLUSIONS

In SUI patients, pelvic support tissue becomes weakened with age, and has a stronger correlation with nNOS containing nerves. However, the underlying neuropathophysiology of SUI is still unclear. The lack of western blotting analysis is a limitation of our study. Well designed further studies are needed to comprehend the role of nNOS pathways in SUI pathophysiology.

CONFLICT OF INTEREST

None declared.

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