ORIGINAL RESEARCH

Changes in Vaginal Physiology of Menopausal Women with Type 2 Diabetes

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ABSTRACT

Introduction. Metabolic disorders, such as type 2 diabetes, have been associated with an increased risk of development of female sexual dysfunction (FSD). In experimental studies, vascular, neuronal, and hormonal responsiveness alteration at vaginal level were proposed as contributors to the onset of FSD in women with diabetes; however, conclusive data on humans are still lacking.

Aims. The study aimed to assess changes in vascularization, sex steroid receptors, nitric oxide synthase, and aquaporin-2 (AQP2) expression occurring at vaginal level in women with diabetes.

Methods. Vaginal biopsies were obtained from 21 postmenopausal women, 10 of whom were diagnosed as having type 2 diabetes mellitus. CD31, estrogen receptor-α (ERα) and androgen receptor (AR) expression and localization were analyzed by immunostaining. Expression of endothelial (eNOS) and neuronal (nNOS) nitric oxide synthase isoforms and AQP2 were also assessed in vaginal samples.

Main Outcomes Measures. Changes in vaginal vascularization, sex steroids receptor, eNOS, nNOS and AQP2 expression.

Results. Vaginal samples from women with diabetes showed an increased microvessel density in the lamina propria, which were morphologically disrupted suggesting an angiogenic compensatory mechanism. While no differences were seen in ERα, AR expression was significantly reduced in the vaginal epithelium and lamina propria of women with diabetes. Similarly, the gene and protein expressions of both nNOS and eNOS were significantly reduced in patients with diabetes, while AQP2 mRNAs level did not significantly differ between the two groups.

Conclusion. Diabetes greatly impacts vaginal physiology, being associated with alterations of the vaginal lamina propria vascular network, nitrergic signaling, and AR expression. These alterations may contribute to the increased risk of FSD development in women with diabetes. **Baldassarre M, Alvisi S, Berra M, Martelli V, Farina A, Righi A, and Meriggiola MC. Changes in vaginal physiology of menopausal women with type 2 diabetes. J Sex Med 2015;12:1346–1355.**

Key Words. Vagina; Type 2 Diabetes; Female Sexual Dysfunction (FSD); Vaginal Vascularization; Sex Steroids Receptors; Nitric Oxide Synthase; Aquaporin-2

Introduction

Female sexual function is the result of the interplay of complex physiologic, psychologic, social, and cultural stimuli, which can affect various aspects of sexual response such as desire, arousal, lubrication, and orgasm [1]. The vagina plays a key role in enhancing desire and arousal through the

transmission of sensations, ensuring adequate lubrication during sexual intercourse, thus preventing pain and also by demonstrating profound modifications during orgasm [1]. The vaginal wall consists of four layers, namely the stratified squamous cell epithelium, the lamina propria, the muscularis, and the tunica adventitia [2]. After sexual stimulation, an increased amount of neurotransmitters, including nitric oxide (NO), are released at a vaginal level, thus mediating vascular and nonvascular smooth muscle relaxation, increasing vaginal blood flow and sub-epithelial capillary inflow, thus leading to vaginal transudate [3]. Changes in sexual function occur during various phases of a woman's life. Menopausal status is associated with changes in vaginal physiology, thus contributing to the changes in sexual function that are present in postmenopausal women [4,5].

Several authors have suggested that diabetes negatively impacts on women's sexual life [6,7]. Indeed it has been reported that diabetes affects almost all domains of female sexual function eventually leading to a reduced Female Sexual Function Index in women with diabetes compared with healthy controls [8]. Although the pathophysiologic substrate of female sexual dysfunction (FSD) in women with diabetes has not been fully understood to date, microvascular and neuronal alterations as well as tissue remodeling may play a role.

In animal studies, diabetes greatly impacts vaginal physiology being associated with reduced sex steroid receptor expression, namely estrogen receptor-α (ERα), progesterone receptor and androgen receptor (AR) [9,10]. Alterations in sex steroid receptors were accompanied by an altered hemodynamic response and nitrergic signaling. Aquaporin water channel (AQPs) expression was significantly reduced in vaginal samples from diabetic rats, resulting in lower vaginal fluid secretion following pelvic nerve stimulation [11,12].

No morphologic and molecular studies have been performed in the human vagina in order to confirm the biologic mechanism demonstrated in animal studies. The aim of the present study therefore was to assess changes in vaginal physiology, including sex steroid receptor expression, vascularization and NO synthase (NOS) expression at the vaginal epithelium and lamina propria levels, occurring in menopausal women affected by type 2 diabetes mellitus compared with menopausal women without diabetes.

Materials and Methods

Study Population

Women were screened for study enrollment from those attending our clinic for scheduled surgical procedures (benign endometrial polyps, adnexectomy for benign ovarian masses, transvaginal sling placement for urinary incontinence). Diagnosis of menopause was carried out according to the definition of the American College of Obstetricians and Gynecologists [13]. Inclusion criteria were permanent amenorrhea for at least two years, absence of major medical illnesses including oncological problems based on medical history and routine laboratory tests, together with no hormonal treatment for at least 1 year. The study protocol was approved by the Human Subject Committee of S. Orsola—Malpighi University Hospital and written informed consent was obtained from all women in accordance with the 1975 Declaration of Helsinki.

Study Design

At study inclusion, clinical, biochemical, hemodynamic, and anthropometric parameters were recorded. Diagnosis of diabetes was carried out according to the American Diabetes Association Guidelines [14]. Hormonal assays, plasma glucose concentrations as well as other routine biochemical evaluations were performed by the centralized clinical laboratory of the S. Orsola—Malpighi University Hospital. Glycated hemoglobin and insulin concentrations were only available for patients with diabetes.

Tissue samples were collected during surgery from the proximal anterior and distal anterior vaginal wall. Specimens measured approximately 1 cm by 1 cm and included both the vaginal epithelium and lamina propria. After withdrawal, each vaginal specimen was divided into two portions, one was frozen for total RNA and protein extraction while the other was fixed in 10% phosphate-buffered formalin for immunohistochemical analysis as previously described [15].

Immunohistochemistry

Vaginal tissue samples of four microns were deparafinized and subjected to antigen retrieval according to primary antibody datasheet instructions. After incubation in 10% normal goat serum for 45 minutes, sections were stained overnight at 4°C with mouse monoclonal anti-ERα antibody (1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA), mouse monoclonal anti-AR antibody (1:50;