



Study of Vaginal Tissue For Collagen Content, Elastin, Fibroblast in Genital Prolapse

KEYWORDS

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Introduction:

Normal physiological function of the pelvic organ depends on the anatomic integrity of the support components. Pelvic support defects may contribute to pelvic organ prolapse; urinary and anal incontinence; sexual; voiding and defecatory dysfunctions. Pelvic support anatomy has been a subject of debate for over a century. Particularly controversial has been the relative contribution of the connective tissue and levator ani muscles. 1,2 Consequently many inconsistencies in terminology are found in literature describing pelvic floor muscles and connective tissue.

The connective tissue of the pelvis is comprised of collagen, elastin, smooth muscle and micro fibers which are anchored in extracellular matrix polysaccharides. There is evidence that suggest abnormalities of connective tissue and connective tissue repair may predispose women to prolapse. For example, Norton found that women with joint hypermobility had a higher prevalence of genital prolapse (cystocele, rectocele and uterine/vaginal vault prolapse) compared with women with normal joint mobility. Women with connective tissue disorders such as Ehlers-Danlos or Marfan's syndrome are more likely to develop pelvic organ prolapse³⁵ and urinary incontinence. 3,4 One third women with Marfan's syndrome and three fourths of women with Ehlers-Danlos syndrome reported a history of pelvic organ prolapse.⁵

Abnormalities in the anatomy, physiology and cellular biology of smooth muscle in the vaginal wall may contribute to the pathophysiology of pelvic organ prolapse. For example smooth muscle fibers arising from the vaginal wall attach to the levator ani complex and dysfunction of this smooth muscle may affect the attachment of lateral vaginal to pelvic wall. Additionally it has been shown that the fraction of the smooth muscle in the muscularis of the anterior and posterior vaginal wall apex in women with prolapse. 6,7 A decreased smooth muscle content of the round ligament in women with pelvic organ prolapse has also been described.

The cellular processes that affect these changes in the vaginal wall during pathogenesis of pelvic organ prolapse are unknown. Currently it is not known whether changes in smooth muscle content are a result of the mechanical forces imposed on the prolapsed tissues, or if decreased amounts of muscularis smooth muscle have a role in development of this disorder. Decreased content of differentiated smooth muscle in the vaginal wall of women with pelvic organ prolapse may be secondary to mechanical forces imposed on prolapsed vaginal

tissues or to denervation of the vaginal tissues during vaginal delivery. 8 Nevertheless, decreased fraction of smooth muscle in the muscularis of prolapsed vaginal tissue may impair vaginal tone. So the aim of the study to prove that loss of collagen, fibroblast and elastin on vaginal tissues specimens as a loss of integrity & to prove the connective tissue fibers play a probable etiological role in genital prolapse in all age group women.

Materials & Methods: This study was conducted in Madha Medical College and Hospital in the department of Obstetrics and Gynaecology during the period of November 2014-November 2015; included all the cases of genital prolapse undergoing surgery (Vaginal Hysterectomy; cystocele undergoing cystocele repair rectocele undergoing Pelvic floor repair and Vaginal hysterectomy with or without cystocele repair, with or without enterocele repair) vaginal tissue was collected from the anterior vaginal wall, posterior vaginal compartment and supporting ligaments such as cardinal ligaments, pubocervical and uterosacral ligaments.

50 cases of women with genital prolapse who underwent surgical procedure in RMMC&H were randomly selected. Patient who underwent vaginal hysterectomy AND/ OR repair of anterior/ Posterior compartment. All genital prolapse cases with age of reproductive, perimenopausal, menopausal with oestrogen deficiency included in the study. Tissue taken from the vaginal mucosal epithelium and also tissues from the ligamentous support of the genital organs.

Patients selected were admitted in Gynaec ward and a brief history and physical examination was done. Patient underwent routine blood investigations, urine routine and worked up for surgery. Before surgery an informed written consent was taken from all patients for obtaining anterior vaginal wall, posterior vaginal wall and pelvic floor connective tissue samples for the study. After surgery specimens were collected in 5% formalin and histological study was done for all samples. In histology mainly the concentration of collagen and type of collagen, concentration of elastin and fibroblasts were studied from the vaginal tissue. Collagen fibre orientation was analysed by scanning electron microscopy.

All women with genital prolapse with cystocele alone Enterocele with or without rectocele & all degree uterine descent were included in the study. Patients with decubitus ulcer, Irreducible genital prolapsed, associate vaginitis, cervical Pathology, were excluded from the study

Results :

According to my study on analysing all histological specimens of anterior vaginal wall, connective tissue from pelvic floor, posterior vaginal wall and enterocele sac it was found that various factors were responsible for prolapse.

There were 8 cases of reproductive age group women who presented with prolapse (16%) and 27 cases of premenopausal age group (54%) and 15 cases of postmenopausal age group (30%). When all the cases were grouped at an age interval of 6 years there were 4 cases between the age group of 24-30 years (8%), 4 cases between age group of 31-35 years (8%), 7 cases between the age group 36-40 years (14%), 7 cases between age group 41-45 years (14%), 13 cases between age group 46-50 years (26%), 15 cases of age group >50 years (30%). There was an increase of collagen.

Fibrils mainly type III collagen in women of reproductive age group. Mainly this increased expression of collagen type III was due to the tissue that is actively remodelling under the biomechanical stresses associated with prolapse. The amount of total collagen in vagina was increased in women with prolapse relative to without prolapse. In women of premenopausal age group there was loss of collagen; fibroblast and elastin but in postmenopausal women there was loss of integrity of connective tissue due to lack of oestrogen. Women of reproductive age group who presented with genital organ prolapse were attributed to connective tissue disorders or neuropathic disorders like Ehler Danlos Syndrome.

In my study there was one case of prolapse belonging to P1 (1%). There were 12 cases of prolapse between P2-P3 (24%). There were 24 cases of prolapse between P4-P5 (48%). There were 13 cases of prolapse reported with P>5 (26%).

In my study maximum number (36%) of cases of prolapse came from the group who had less than 1 year spacing between pregnancy followed by the group who had spacing between 1- years (32%). The least number of cases was reported from the group who has spacing between -3 years (14%). It was also found that women had spacing of less than 1 year between deliveries had repeated injury of the pelvic connective tissue and muscles which probably attributed to genital organ prolapse at a comparatively earlier age when compared to those women of the same age who had adequate spacing.

Maximum (62 %) of cases of prolapse were reported in women who had normal vaginal delivery which caused overstretching of the perineal muscles. 30% of women who had prolapse had both normal and instrumental delivery. 6% of women had prolapse had both normal and operative delivery. 2% of women had prolapse had all three modes of delivery (normal+instrumental+operative).

24% of cases underwent vaginal hysterectomy with anterior colporaphy with posterior colporaphy; 18% of cases underwent Posterior colporaphy; 16% of cases underwent Vaginal Hysterectomy; 12% of cases underwent Vaginal Hysterectomy with Anterior colporaphy; 10% of cases underwent with anterior colporaphy; 6% of cases underwent both trial Vaginal Hysterectomy and Enterocele repair; 2% of cases underwent vault repair with posterior colporaphy;

44% of cases had increased Type 1 collagen; 20% of cases had decreased Type 1 collagen; 16% of cases had Type 1

collagen of unchanged quantity. About 54% of cases had increased Type 3 collagen; 30% of cases had decreased Type 3 collagen; 16% of cases had Type 3 collagen of unchanged quantity. Type 2 collagen was not observed in any of my specimens. 68% of cases had decreased Elastin and about 70 % of cases had increased Fibroblast content and 48% of cases had decreased Smooth muscle content.

In this study 44% of postmenopausal women were supplemented with oestrogen showed an increase in Type I collagen and 40% of postmenopausal women with no oestrogen supplementation showed an increase in collagen Type III. In my study some postmenopausal women were supplemented with exogenous oestrogen that showed their connective tissue had a slightly content of collagen; fibroblast and elastin.

DISCUSSION

To evaluate the appropriateness of pelvic muscle training of a woman, muscular strength, duration of contraction and extent of displacement of the examining fingers is assessed. Although not standardised, a scale commonly used to assess pelvic floor strength.⁹

A women's ability to sustain a pelvic contraction a minimum of seconds is likely to ensure a better response to physiotherapy. Displacement of the examiner's finger in an inward or upward direction into the vagina is also a physical finding likely to be associated with normal structural attachments of the pelvic muscles as well as intact sensory muscle and motor innervations.^{10,11} A patient on repeat attempts is unable to perform 1/5 muscle contraction is likely a poor candidate for PMT and requires more intensive pelvic floor therapy such as transvaginal electrical stimulation therapy.

Patients with pelvic floor prolapse should be counselled on the importance of various lifestyle modifications that may prevent or improve their symptoms of prolapse. Maintaining an ideal body weight limits the pressure that the abdominal content places on the pelvic floor. Any activity such as walking or gardening that engages the pelvic floor can help strengthen the muscles. Patient should be counselled to squeeze or perform a Kegel contraction when lifting or straining. Physical therapists also recommend discussing bowel habits with patients because bowel movement retraining will teach a passing motion without straining the pelvic floor muscles.¹²⁻¹⁶

According to ACOG 2005 conducted a survey between premenopausal and postmenopausal women and analysed amount of collagen in the vaginal tissues and found that the amount of total collagen in the vagina was increased in women with prolapse relative to women without prolapse primarily due to increased expression of collagen III.^{17,18} This increase in collagen III and active matrix metalloproteinases MMP-9 expression in the vaginal tissues of patients with prolapse suggests that tissue is actively remodelling under the biomechanical stresses associated with prolapse. In my study 54% of case had an increase in total collagen which mainly was contributed by type III collagen. According to BJOG 2005 et al conducted a study to compare tissue markers of collagen metabolism in the uterosacral ligaments and vaginal tissue of women with prolapse and found that there was significant increase in pro matrix metalloproteinases (MMP) 9 in all prolapse cases.

According to Acta Obstetricia et Gynaecologica Scandinavica 15 conducted as study by taking biopsies from

vaginal tissue and pelvic floor connective tissue from prolapse patients and analysed that there was a decreased elastin content in all prolapse cases when compared to women without prolapse. Elastin was measured by calculating the mean desmosine concentration. In patients who had more vaginal deliveries had a greater decrease in elastin content. It suggests that there is an altered elastin metabolism in prolapse cases. In my study 68% of women had decreased elastin content in the vaginal tissues. According to ACOG 2005 conducted a study comparing vaginal myofibroblast content in prolapse cases found that myofibroblasts of women with uterovaginal prolapse are poorly contractile and there is a decreased myofibroblast content when compared with that of women without prolapse. In my study 70% cases had decreased fibroblast content.

According to ACOG 2005 studied the effect of estrogen on collagen synthesis by measuring the expression of collagen I and collagen III mRNA and found that Estrogen increases collagen gene transcription and indicates stimulation of collagen synthesis in pelvic floor connective tissue. 16,17

According to Current opinion in Obstetrics and Gynaecology et al conducted a study to compare the smooth muscle content of the anterior vaginal wall in normal women and women with pelvic organ prolapse and found that smooth muscle content was most diminished in specimens from postmenopausal women with no estrogen replacement whereas smooth muscle was also decreased significantly in premenopausal women with prolapse. Therefore smooth muscle content in vaginal wall is significantly decreased in women with pelvic organ prolapse compared with normal subjects. In my study about 6% of postmenopausal women supplemented with oestrogen had decreased smooth muscle content. 40% of postmenopausal women who were not supplemented with oestrogen had decreased smooth muscle content.

According to ACOG 2004 et al conducted a study to determine the impact of menopause on the structural components of Arcus tendineus fasciae pelvis and found that ATPF was comprised primarily of collagen bundles of Type III collagen fibers (84%) and an intermediate amount of elastin (13%) and very little smooth muscle. The ratio of collagen I/(III+V) was decreased in postmenopausal not on hormones relative to premenopausal women due to 75% decrease in collagen I. The decrease in collagen I and change in collagen ratios was not present in women on hormone therapy. 18,19 Menopause in the absence of hormone therapy was associated with a decrease in quantity of collagen I in the ATPF resulting in a decrease in ratio of collagen I/(III+V). This may compromise the tensile strength and increase susceptibility to pelvic organ prolapse. In my study ratio of collagen I/(III+V) in all postmenopausal women with no estrogen supplementation was about 0.8 whereas the ration in postmenopausal women with oestrogen supplementation was 1.14 which was already proved by ACOG 2004 study.

Genital prolapse is one of the most common findings in outpatient Gynaecology department. Early detection of prolapse is difficult in country like ours because of the large population and most of them belonging to low socioeconomic status who do not come for regular gynaec checkups. Women with pelvic organ prolapse can present with a wide variety of bladder: bowel and pelvic symptoms. With the exception of vaginal bulging symptoms,

however; none are specific to prolapse. There is considerable overlap with other pelvic floor disorders and clinicians should be cognizant of other potential important landmark for symptom development for pelvic organ prolapse (POP Q) quantification. When pelvic organ prolapse extends beyond the hymen; the number of symptoms and degree of bother caused by these symptoms will be more for the patient. 20 Additionally, the nature of the symptoms can change, For instance, in women with prolapse beyond the hymen, voiding dysfunction symptoms are more prevalent.

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