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The Characteristics of the Connective Tissue of the Pelvic Floor at the Collapse of the Genitals

ABSTRACT

The objective of our study is to clarify the distribution of immunohistochemical marker expression of MMP-1, MMP-2 and TIMP-1 in the sacrouterine and round ligaments of the uterus among women with genital prolapse.

The research was performed on the material of biopsies of sacrouterine and round ligaments of the uterus on both sides taken during vaginal hysterectomy from 15 women with genital prolapse aged 42 to 65 years old. It was found that the decrease in the accumulation of TIMP-1 on the background of high expression of metalloproteinases in the POP contrasts with a moderate level of expression of MMP-2, low MMP-1 and moderate TIMP-1 in the control group, which affects the density of the VCR. Connective tissue loosening, reducing of its elasticity and tensile strength was accompanied by degradation of fibers and increased degradation of collagen, defining pathobiochemical disorders in the pelvic dysfunction.

Keywords: genital prolapse, matrix metalloproteinases, tissue inhibitors of matrix proteinases.

INTRODUCTION

The problem of genital prolapse which is considered to be crucial due to increasing morbidity and severe dysfunction of pelvic organs seems not only medical, but also a serious social problem [2,4]. Dysfunction of pelvic organs, combined with incomplete emptying of the bladder and the bowel, which manifests itself in a variety of symptoms - feeling of heaviness and a foreign body in the lower abdomen, dysuria and dyspareunia - causes patients significant physical and mental suffering.

Hidden epidemic - that is the definition that has formed about the problem of pelvic floor failure not only because of the prevalence of older women segment among patients and, according to foreign researchers, because of their highly active way of life. There is also a forecast about doubling the number of requests for medical help in the next 30 years [2].

We have to mention a pessimistic conclusion about the growth rate of genital prolapse among women of reproductive age and poor quality of life that require surgical correction. Despite the vast amount of methods of surgical treatment of pelvic floor dysfunction, none of

the practiced techniques can be considered stable and effective due to frequent statements of recurrence of the disease, followed by a succession of plastic interventions and partial or total patients' disability.

According to literature, the need for repeated surgical treatment of genital prolapse occurs in one third of cases, reaching the anatomy of the pelvic floor 61.3% within a period of three years after the rapid restoration [2]. These facts point to the need for in-depth study of aspects of etiology and pathogenesis of the disease, for which there is still no consensus [14]. Alongside with contradictory and ambiguous judgments, debates about the damage of any single anatomical structure, which determines the lack of fixation of pelvic organs, are constantly going on. We discuss the existence and nature of damage to the muscle layer as a key factor in the development of genital prolapse and pelvic floor failure accompanied by isolated or combined dysfunction of pelvic organs. This theory, first advanced by J. Halban and J.Tandler in 1907, disagrees with the teachings of Schultze (1881) about the leading in the development of omission, and then prolapse failure supporting ligaments, namely - the round ligament, according to a further submission - retaining ligaments - cardinal and sacrouterine. However, at the present stage of medicine development the idea emerged that taking into account ensuring of the fixing process and the normal functioning of pelvic tissues of different "phenotypes" - connective tissue component and striated muscle, their involvement in genital prolapse development is sure to be mutual.

Recent-year studies have allowed to study the genesis of genital prolapse from the standpoint of the role of molecular-biological factors [11]. Development of the theory of connective tissue dysplasia (CTD) - states with different clinical manifestations of this disease, with certain congenital viscerotomy lesions arising embryonically and postnatally, with progressive course and a number of dysfunctional manifestations, has defined a new direction of scientific research in the perineology sphere [3]. Due to the conclusion of a multi-organ involvement in CTD and metabolism disorder - a defect of synthesis or degradation of extracellular matrix (ECM) and dysregulation of morphogenesis structures of the pelvic floor, tissues and their histological and immunohistochemical features were the subject of careful study at genital prolapse [1].

It is obvious that the manifestations of pelvic dysfunction such as stress incontinence and genital prolapse may have a common pathophysiological basis due to violation of the anatomical

relationships and unspecified changes in the functional activity of the pelvic floor structures at not only the macro- and micro-levels.

The basis of connective tissue ECM is presented by gel medium elastin and collagen fibers [3]. The content of collagen and elastin - the main proteins of connective tissue in genital prolapse - varies dramatically. Type I collagen can be found in bones, scars, tendons, cartilage, II is the main component of cartilage, III is found in reticular fibers, IV prevails in subepithelial layer and in basal membrane. Elastic fibers provide flexibility and elasticity of tissues; collagen contributes to their strength and integrity. [10] According to data, patients with genital prolapse have violations of the ratio of their content. Due to lower total collagen and elastin the number of fractions of crude, more "fragile" collagen increases [6,13].

Having taken into consideration the current knowledge about the molecular and biological mechanisms of genital prolapse it is important to study matrix metalloproteinases (MMPs) - enzymes performing the degradation and remodeling of connective tissue with high biological activity by regulating homeostasis VCR expression during wound healing in normal and pathological cases [4, 12].

MMP-1 is synthesized by fibroblasts and connective tissue monocytes and is involved into catabolism of fibrillar and non-fibrillar collagens, mainly in the supporting apparatus of pelvic organs [7]. MMP antagonists include proteins tissue inhibitors of matrix proteinases (TIMP), expressed in the tissues of pelvic organs to preserve the dynamic balance [15]. Despite the small number of messages, there is evidence of excess activity of specific MMPs involved in the collagen tissues decay process, not compensated by TIMP, among patients with genital prolapse [5]. It is believed that the contribution of the increase in MMP collagenolysis may be associated with features of genetic polymorphisms, mutant forms of their predominant among women with genital prolapse. [13] However, complicated biochemical interactions of enzymes, proteins and molecules still have to be recognized.

The increasing interest of experts to study the importance of ECM components imbalance - MMP and TIMP - in the genesis of pelvic floor failure can be explained not only by the possibility of clarifying the molecular mechanisms predisposing to the disease, but also by prediction the likelihood of relapse.

The objective of our study is to clarify the features of the distribution of immunohistochemical marker expression of MMP-1, MMP-2 and TIMP-1 in the sacrouterine and round ligaments of the uterus among women with genital prolapse.

MATERIALS AND METHODS

The research was performed on the material of biopsies of sacrouterine and round ligaments of the uterus on both sides taken during vaginal hysterectomy from 15 women with genital prolapse aged 42 to 65 years old. The control group was presented by biopsies of ligaments (sacrouterine and round) from 15 women without genital prolapse aged 35 to 52 years old who also underwent abdominal hysterectomy. 4 samples of tissue were obtained from each patient: right and left sacrouterine ligaments and right and left round ligaments of the uterus. Biopsies were fixed in 10% neutral formalin and embedded in paraffin, and then paraffin sections were made 4 microns thick. The samples were stained with hematoxylin and eosin, with picrofuchsin according to Van Gieson and fuchselin according to Weigert. Additional non-stained sections on polylysine glass were produced and immunohistochemical reactions by the standard method with thermal unmasking of antigens using primary antibodies to MMP-1 and MMP-2 (LabVision, readytouse), TIMP-1 (LabVision, 1:50) were carried out.

The results of immunohistochemical reactions were scored according to the percentage of stained cells or the percentage of stained VCR.

Mathematical processing of the obtained results was carried out by methods description and a parametrical statistics on a personal computer with the aid of the program «Statistics 7.0».

RESULTS AND DISCUSSION

According to the information received, COP and CCM expression of MMP-1 and MMP-2 was observed in the form of lumps of brown staining in the extracellular matrix, fibroblasts, ligament apparatus and in vascular endothelium within pelvic connective tissue structures. A similar tendency was observed in connection with marker TIMP-1 visualization (Fig.).

Ligamentous apparatus among patients with genital prolapse can be characterized by increased levels of metalloproteinase activity in comparison with indicators in the group of healthy women. Expression of MMP-1 in genital prolapse turned out to be twice as big (4± 1.2), compared to the rate in the control group (2 ± 0.8). The expression level of MMP-2 in genital prolapse was 6 ± 0.3 points, which is half as significantly higher than the rate of enzyme accumulation among healthy women (4 ± 0.5 points). The increase of metalloproteinase activity in genital prolapse went along with decreased expression of the natural inhibitor TIMP-1, the level of which amounted to 1.5 ± 0.5 points versus 4 ± 0.7 points in the control group.

Thus, the reduction of TIMP-1 accumulation alongside with high expression of metalloproteinases in genital prolapse contrasted with moderate level of MMP-2 expression, low

MMP-1 and moderate TIMP-1 within a control group. It affected the density of VCR as well. Connective tissue loosening, reducing of its elasticity and tensile strength was accompanied by degradation of fibers and increased degradation of collagen, defining pathobiochemical disorders in the pelvic dysfunction. Preservation of tissue homeostasis among healthy women was observed on the background of high content of metalloproteinase TIMP-1 inhibitors and a slight but optimal for maintaining tissue strength expression level of MMP.

The results obtained agree with the data concerning the important role of violation of dynamic equilibrium processes of synthesis, postranslation transformation and IMF and SST degradation in the tissues of the ligamentous apparatus - COP and CCM among patients with genital prolapse. The presence of misbalance between MMPs and their inhibitors, initiating remodeling of the ECM in the ligaments of the uterus in genital prolapse is proved by the works of other studies [7, 10]. Increase in the expression of MMP-1, MMP-2 and MMP-9 in the CCM and vaginal tissues is reported to be found among patients with genital prolapse as compared to the control [9, 12]. Conclusions about overall excess of MMP activity and significant TIMP decrease in vaginal tissue among white women with premenopausal genital prolapse present vivid evidence of the violation of tissue metabolism components of connective tissue and the VCR [8].

CONCLUSION

The research conducted proves a significant role of connective tissue in fixation and normal functioning of pelvic organs. It also points out that mechanical properties of the ligament apparatus are determined by the level of proteolytic enzymes expression. We put forward the idea about pathogenetic importance of degradation and synthesis activity of connective tissue components in genital prolapse. Identified molecular biological processes of tissue remodeling are accompanied by changes in the activity of enzyme complex ECM and allow to considerimmunohistochemical diagnostics as widening of predictive capability referring to risk of recurrence of the disease after surgery. Continued research in this area is promising for the development of innovative reconstructive surgery and preventive measures to reduce the risk of genital prolapse relapse.

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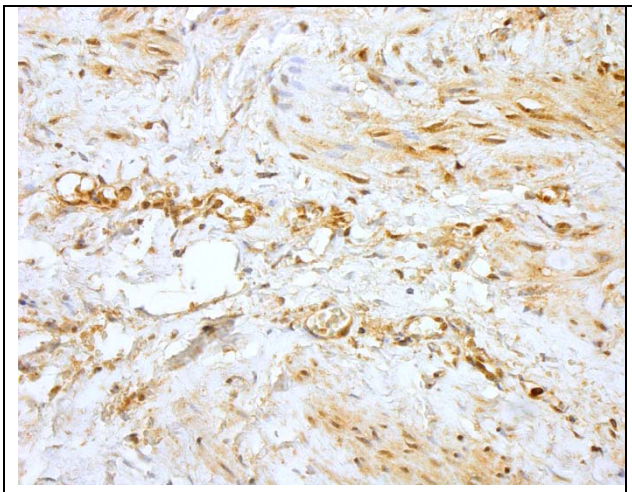

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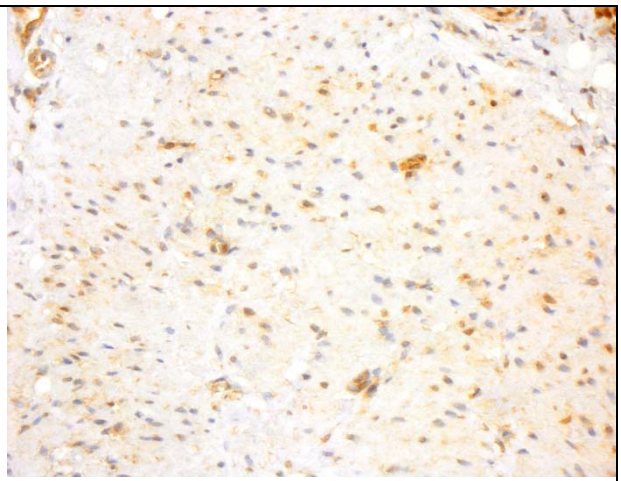

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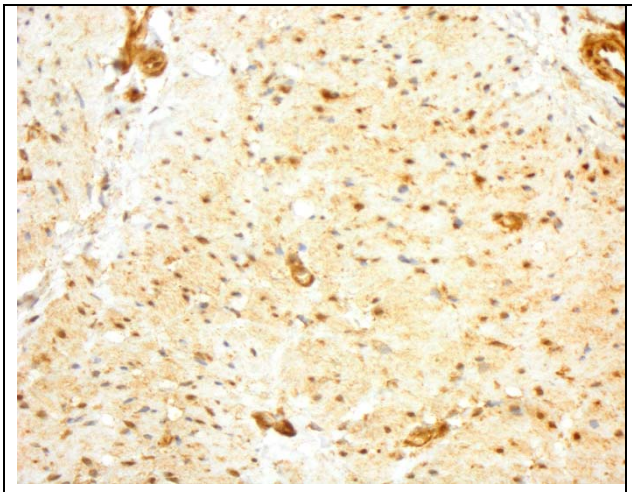

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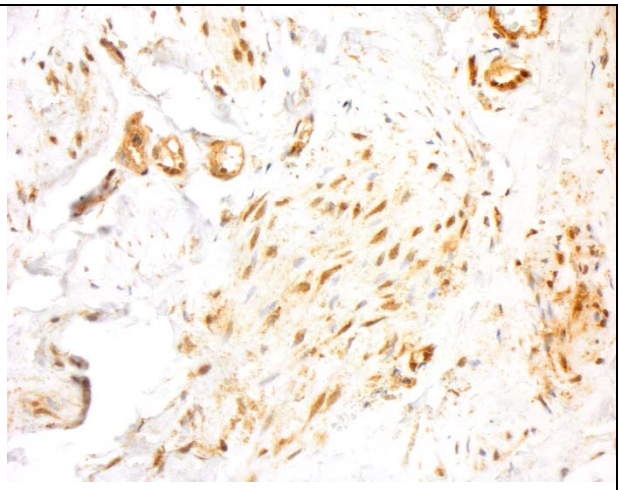

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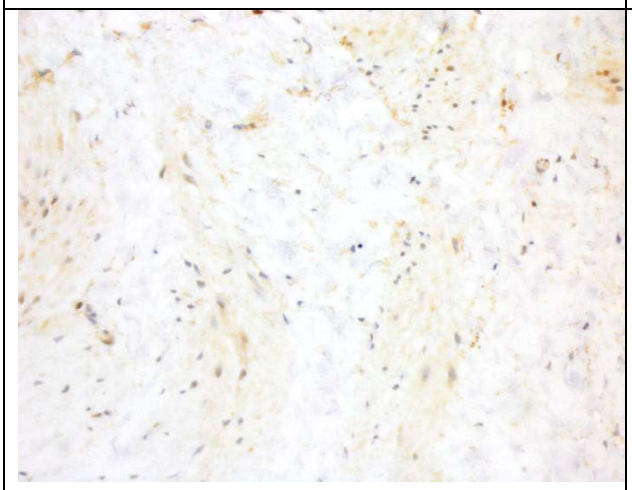

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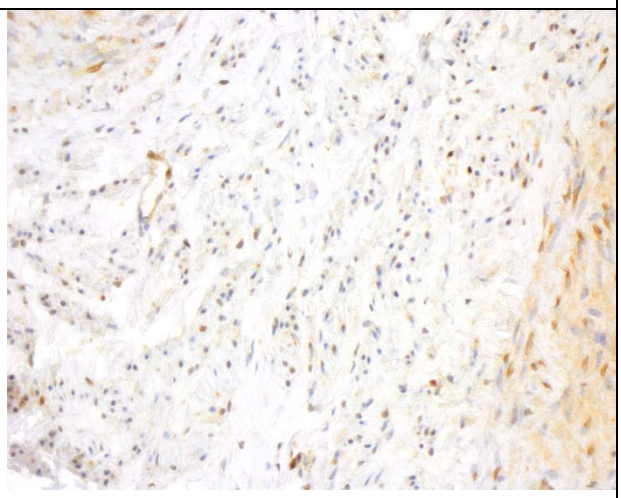

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Figure. MMP-1 MMP-2 TIMP-1in round ligaments of the uterus, *400:main group (aMMP-1, c MMP-2, e TIMP-1), control group (b MMP-1, dMMP-2, f TIMP-1).