EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Serum Activity of Matrix Metalloproteinases -2 and -9

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Matrix metalloproteinases belong to the key effectors of tissue remodeling in health and disease. Matrix metalloproteinases -2 and -9, the most prevalent representatives of this family, are expressed in the endometrium. Chronic endometritis concomitant with sterility and spontaneous abortions is associated with decreased content of matrix metalloproteinases in the endometrium. Chronic endometritis combined with sterility correlates with decreased serum activities of matrix metalloproteinases -2 and -9, which returns to normal after recovery. Measurements of serum activities of matrix metalloproteinases -2 and -9 are proposed for monitoring of the dynamics and treatment efficiency in chronic endometritis.

Key Words: *matrix metalloproteinases; chronic endometritis*

Implantation and development of the placenta are the most intricate processes in developmental biology. Realization of these key reproductive functions requires balanced cooperation between growing body tissues and maternal organism. Matrix metalloproteinases (MMP), expressed by invasive cells of the fetal origin and by endometrial cells, are involved in this cooperation. The MMP family is a group of structurally related zinc-dependent endopeptidases normally destroying the basal membranes (BM) and extracellular matrix (ECM) [5]. They are produced in an inactive state, are transported to the extracellular space, and activated there under the effects of other proteases [10]. The sources of MMP in the endometrium are fibroblasts, vascular wall endotheliocytes, macrophages, and neutrophils [5].

The leading role in ECM remodeling during implantation belongs, among other factors, to MMP-2 and MMP-9 [6,13]. MMP-9 provides controlled degradation of BM, while MMP-2 is responsible for adequate level of basal MMP activity. Disorders in MMP expression in the endometrium are associated with disorders in implantation and sterility. Low level of MMP-2 gene expression in the endometrium is detected in women suffering from habitual miscarriages [4]. After extracorporeal fertilization a significant difference between MMP-2 activity in women with effective conception and its failure can be detected as early as on day 3 after embryo implantation [3]. Chronic inflammatory process in the endometrium is an important cause of sterility, habitual miscarriages, and failure of extracorporeal fertilization [14]. Therefore, study of MMP-2 and MMP-9 expression and activity in chronic endometriosis is an important task.

A relationship between changes in the balance between MMP levels in the serum and pathological focus in diseases of the female reproductive system is demonstrated. In patients with gestosis, activity of MMP-2 increases in the serum [12] and umbilical artery [7]. Preterm delivery is associated with increased concentration of MMP-9 in the amniotic fluid [3] and serum [11]. Endometriosis is characterized by not only typical changes in MMP balance in the eutopic and ectopic endometrium [10], but also shifts in serum MMP-2 activity [9]. Decreased con-

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tent of MMP-9 in the endometrium is fraught with the risk of spontaneous abortion, including that in chronic endometritis [1].

We studied the relationship between serum MMP-2 and MMP-9 activities and chronic inflammatory process in the endometrium.

MATERIALS AND METHODS

The study was carried out in 11 women (mean age 29.1±2.3 years) with chronic endometritis and sterility receiving standard combined etiotropic therapy. Control group consisted of 5 healthy women. The serum was analyzed on days 5-7 of the menstrual cycle before and on days 5-7 of the cycle 2 months after therapy; in controls serum was analyzed on days 5-7 of the cycle.

Serum activities of MMP-2 and MMP-9 were measured by electrophoresis in 8% polyacrylamide gel after Laemmli [15]. The sera were diluted with a buffer (62.5 mM Tris-HCl, 2% sodium dodecylsulfate, 10% glycerol, pH 6.8), applied onto gel containing 8% acrylamide/bis-acrylamide mixture (37.5:1), 0.125 M Tris-HCl (pH 6.8), 0.1% sodium dodecylsulfate) and separated by electrophoresis at 30 mA constant current. The gel was washed (2×30 min) at 20°C in the incubation buffer (5 mM Tris-HCl, 2.5% Triton X-100, pH 7.5), stained with 2% Coumassie blue, washed again, left for 2 h in distilled water at room temperature, and then dried.

RESULTS

The gel after electrophoretic separation of sera from 7 patients with chronic endometritis combined with sterility before etiotropic therapy is shown in Fig. 1 (*1-7*). The gel with sera from other 4 women with chronic endometritis before (*8-11*) and after therapy (*12-13*) is shown in Fig. 2. Activities of MMP-2 and MMP-9 in 3 sera from control group (healthy women) are shown in Fig. 3. Different intensities of white bands in the gels corresponding to MMP-2 and MMP-9 indicate a decrease in MMP activity on days 5-7 of the cycle in the sera from women with chronic endometritis and sterility before therapy, while after clinical and histological recovery these parameters returned to normal.

A decrease in serum MMP-2 activity was observed in women with chronic endometritis combined with sterility (Figs. 1, 2) in comparison with samples from patients after effective treatment and from controls (Fig. 3), which is in line with decreased activity of MMP-2 gene in the endometrium of women suffering from habitual miscarriages [3]. Our

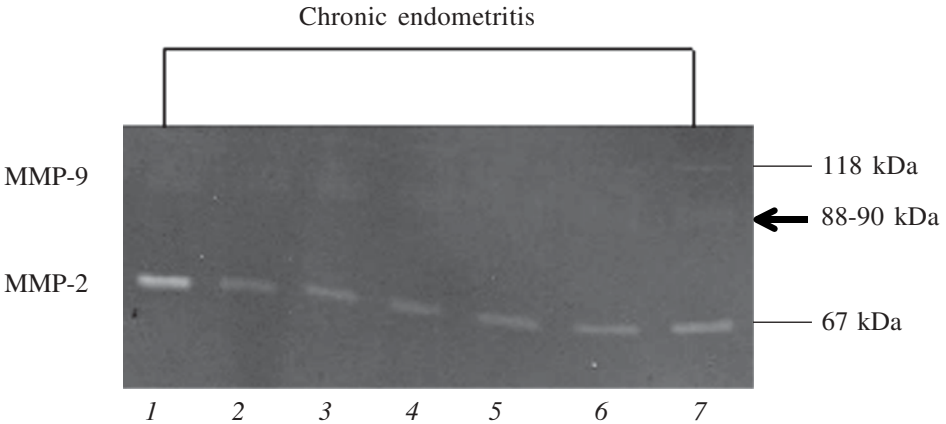

Fig. 1. Serum activities of MMP-2 and MMP-9 (matrix metalloproteinases) in patients with chronic endometritis before therapy (*1-7*). Here and in Figs. 2, 3: white bands at ~67 kDa correspond to MMP-2; arrow shows double bands within ~88-90 kDa, corresponding to MMP-9 and proMMP-9; activity at the level of ~118 kDa corresponds to MMP complexes with other serum proteins.

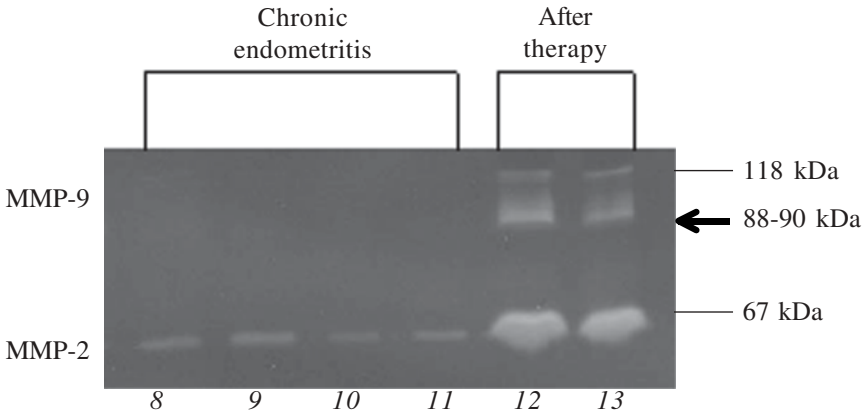

Fig. 2. Serum activities of MMP-2 and MMP-9 in patients before and after therapy of chronic endometritis. *8-11*: before therapy; *12* and *13*: with application of sera from patients Nos. 8 and 9 collected after therapy and histologically confirmed clinical recovery.

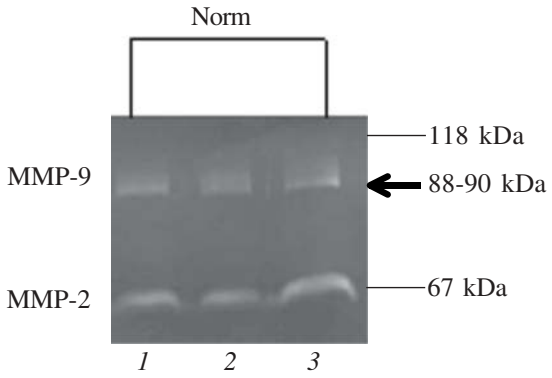

Fig. 3. Normal serum activities of MMP-2 and MMP-9 in 3 healthy women. *1-3*: serum of controls collected on days 5-7 of the cycle.

findings also attest to decreased serum MMP-9 activity in patients, which correlates with the results of immunohistochemical evaluation of MMP-9 in the endometrium of women with habitual miscarriages [1]. Low level of MMP-9 in the endometrium increases the risk of spontaneous abortion, while high level of this protein can serve as a prognostic sign of favorable completion of gestation [1]. Presumably, abnormal activity of serum MMP-2 and MMP-9 in chronic endometritis combined with sterility reflects imbalance of matrix proteins in the endometrium, characteristic of this disease. Normalization of serum MMP activity after therapy corresponds to clinical recovery confirmed histologically and presumably indicates intensive processes of proteolysis of coarse fibrous stromal proteins, typical of chronic inflammation foci, *e. g.*, collagens I and III [5,8,10].

Structural BM proteins are the main substrate for MMP-9 [6,13]. This enzyme is essential for implantation (one of the stages is controlled destruction of BM). Decreased activity of endometrial MMP-9 is a pathogenetic factor indicating impossibility of effective implantation [1]. MMP-2 is a protein whose abnormal expression in the endometrium is associated with sterility and spontaneous abortion [3]. Presumably, changes in serum activities of MMP-2 and MMP-9 in chronic endometritis combined with sterility reflect disorders in the expression these proteins in the endometrium.

Hence, evaluation of the endometrial status by measuring serum protein activities is an alternative to invasive methods for complex diagnosis and monitoring of treatment efficiency in chronic endometritis combined with sterility.

REFERENCES

1. A. V. Kuznetsova, V. S. Paukov, I. N. Voloshchuk, and E. M. Demidova, *Arkh. Patol.*, **1**, 18-22 (2002).
2. M. A. Abdallah, S. T. Nakajima, A. C. Eblen, *et al.*, *Fertil. Steril.*, **82**, 966-967 (2004).
3. N. Athayde, R. Romero, R. Gomez, *et al.*, *J. Matern. Fetal Med.*, **8**, 213-219 (1999).
4. H. K. Choi, B. C. Choi, S. H. Lee, *et al.*, *Mol. Reprod. Dev.*, **66**, 24-31 (2003).
5. T. E. Curry and K. G. Osteen, *Endocrinol. Rev.*, **24**, 428-465 (2003).
6. S. Freitas, G. Meduri, E. Le Nestour, *et al.*, *Biol. Reprod.*, **61**, 1070-1082 (1999).
7. Z. Galewska, E. Bankowski, L. Romanowicz, and S. Jaworski, *Clin. Chim. Acta*, **335**, 109-115 (2003).
8. S. Goldman and E. Shalev, *Front Biosci.*, **9**, 2474-2483 (2004).
9. H. F. Huang, L. H. Hong, Y. Tan, and J. Z. Sheng, *Fertil. Steril.*, **81**, 1235-1239 (2004).
10. D. L. Hulboy, L. A. Rudolf, and L. M. Matrisian, *Mol. Hum. Reprod.*, **3**, 27-45 (1997).
11. E. Makrakis, O. Grigoriu, E. Kouskouni, *et al.*, *J. Matern. Fetal Neonatal Med.*, **14**, 170-176 (2003).
12. H. Narumiya, Y. Zhang, C. Fernandez-Patron, *et al.*, *Hypertens. Pregnancy*, **20**, 185-194 (2001).
13. S. C. Riley, R. Leask, T. Chard, *et al.*, *Mol. Hum. Reprod.*, **5**, 376-381 (1999).
14. A. M. Sharkey and S. K. Smith, *Best Pract. Res. Clin. Obstet. Gynecol.*, **17**, 289-307 (2003).
15. G. Soboleva, B. Geis, H. Schrewe, and B. H. Weber, *J. Cell. Phys.*, **197**, 149-156 (2003).