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The role of the adhesion molecule Nectin-4 in the pathogenesis of endometriosis

R. Bedir[1], I. Sehitoglu[1], G. Balik[2], M. Kagitci[2], H. Gucer[1], C. Yurdakul[1], P. Bagci[3]

[1] Recep Tayyip Erdogan University, Faculty of Medicine, Department of Pathology, Rize
[2] Recep Tayyip Erdogan University, Faculty of Medicine, Department of Obstetrics and Gynecology, Rize
[3] Marmara University, Faculty of Medicine, Department of Pathology, Istanbul (Turkey)

Summary

Objective: Nectins are immunoglobulin-like adhesion molecules, and they play important role in cell proliferation and tumor metastasis. The objective in this study was to compare the expression of Nectin-4 in normal endometrium and in ectopic endometriotic tissues. *Materials and Methods:* Nectin-4 expression was investigated in ovarian endometriosis (n=20), peritoneal endometriosis (n=20), endometrium of endometriosis (n=20), and in a control group (having no endometriosis) (n=20) by immunohistochemical method. *Results:* Nectin-4 expression, when compared with control group, was higher in endometriotic lesions of patients having ovarian endometriosis and peritoneal endometriosis ($p = 0.003$ and $p = 0.009$, respectively). This difference was significant in the endometrium of patients having endometriosis ($p = 0.011$). *Conclusion:* The authors believe that Nectin-4 molecule may contribute to the pathogenesis of endometriosis. For this reason, the use of medicines developed against this molecule in the treatment of endometriosis may be useful.

Key words: Endometriosis; Nectin-4; Immunohistochemistry.

Introduction

Endometriosis is characterized by the presence of endometrial tissues in any region out of endometrium. It occurs in approximately 10% of women in the reproductive period, 5-50% of whom have an infertility complaint, and more than 33% have chronic pelvic pain. Being blood-filled pelvic bulk in general, they are observed in fallopian tubes, Douglas cul-de-sac, uterine ligaments, and rectovaginal septum [1-3]. Extrapelvic localizations include the kidneys, bladder, bowels, lymph nodes, omentum, lungs, pleura, extremities, umblicus, hernia sac, and the abdominal wall [4]. Although the symptoms are wide-ranging, the cardinal clinical findings are chronic pelvic pain and infertility [1].

Nectins are cell adhesion molecules involved in the regulation of epithelial physiology. To date, four types of nectins have been identified. Except for Nectin-4, all nectins are expressed by epithelial, endothelial, hematopoetic, and neural cells in adult tissues. In contrast to other nectin molecules, detection of nectin-4 transcripts is mainly restricted to the placenta in human tissues. Tissue distribution of nectin expression is broader in mouse, and interestingly Nectin-4 is detected at days 11, 15, and 17 during murine embryogenesis [5-7].

The authors' objective in this study was to compare the expression of Nectin-4 in ovarian and peritoneal endometriotic tissues and the endometrium of women with and without endometriosis.

Materials and Methods

The information of the patients diagnosed with endometriosis from 2011-2014 in Recep Tayyip Erdoğan University's Faculty of Medicine, Department of Pathology, was gathered from archived reports. The slides of the cases, which had been stained with hematoxylin-eosin were retrieved from archive and re-evaluated. For the controls, the endometrial tissues of 20 patients, who had biopsy or hysterectomy for other reasons, were utilized. The expression of Nectin-4 was investigated in endometrium of 20 patients with endometriosis, 20 patients with ovarian endometriosis, and 20 patients with peritoneal endometriosis. For immunohistochemical analysis, the slides that clearly showed the endometriosis foci were chosen. Five micron thick serial sections were obtained from the paraffin embedded blocks belonging to the selected cases, fixed with formalin on positively charged slide to study Nectin-4 levels by immunohistochemical (IHC) examination. Anti-Nectin-4 primary antibody was utilized. The biotin-free, HRP multimer-based, hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen containing DAB detection kit and a fully automated immunohistochemistry staining device were used as the IHC staining system. The sections were counterstained with Mayer's hematoxylin. Known positive controls were also stained simultaneously. The results were interpreted by two independent pathologists by means of light microscopic observation.

Immmunostaining assessment

The score was the average of ten distinct high power fields observed at x400 magnification. Cytoplasmic and membranous staining for Nectin-4 was considered positive. Nectin-4 were scored on a scale of 0 to 3: 0 (no staining), 1 (staining of less than 10% of cells = weak staining), 2 (11–50% of cells = moderate to strong staining), and 3 (more than 50% of cells = strong staining).

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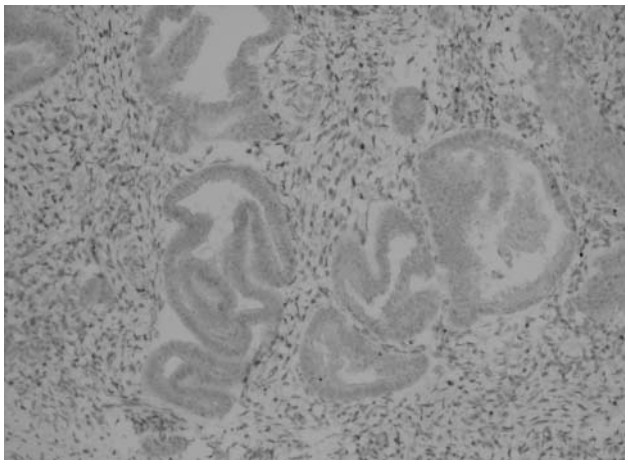

Figure 1. — Negative expression is observed for Nectin-4 in the normal endometrium (immunostaining x200).

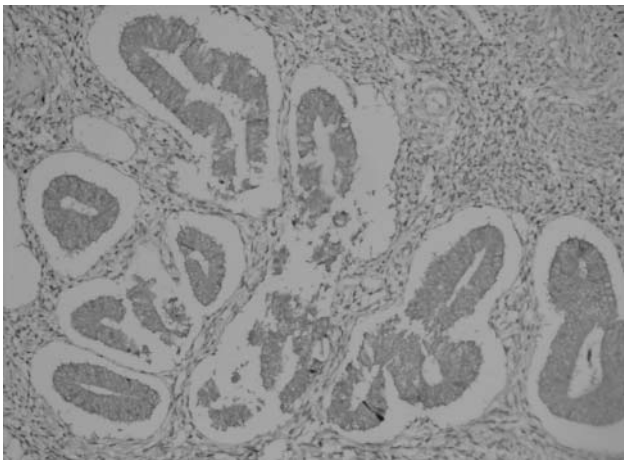

Figure 2. — Weak positive expression is observed for Nectin-4 in the normal endometrium. (immunostaining x100).

Table 1. — *Comparison of nectin staining of endometriosis and control groups. (p1: Chi-square test p2: Kruskal Wallis test).*

Groups	Nectin staining				*p*	
	0	+	++	+++	*p*1	*p*2
Control	5	9	6	0		
All endometriosis groups	1	13	30	16	0.00	0.00
Control *vs.* endometrium of patients with endometriosis	1	4	9	6	0.011	0.001
Control *vs.* ovarian endometriosis	0	4	10	6	0.003	0.00
Control *vs.* peritoneal endometriosis	0	5	11	4	0.009	0.001

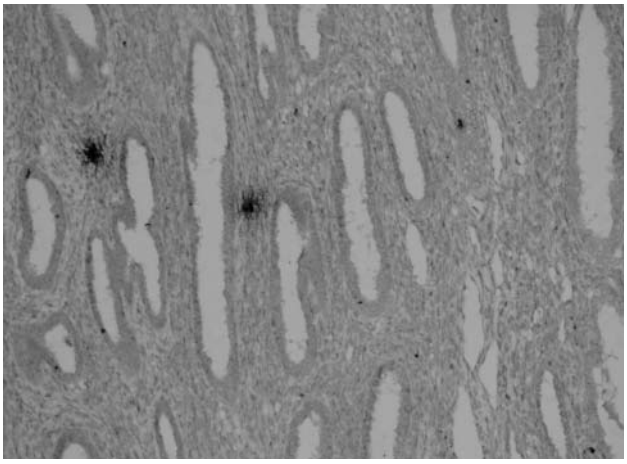

Figure 3. — Moderate positive expression was observed for Nectin-4 in the normal endometrium (immunostaining x200).

For the purposes of statistical analysis, scores of 1, 2, and 3 were considered positive and a score of 0 was considered negative [7].

Statistics

The data were analysed by using SPSS (Statistical Package for Social Sciences) 17.0. Kruskal Wallis test, Mann-Whitney U test, and Chi-Square tests were used to compare variables. A p value of < 0.05 was accepted as statistically significant.

Results

A total of 80 samples were evaluated. Twenty of these belonged to patients without endometriosis acting as controls and 60 belonged to patients with endometriosis. Each of the three study groups had 20 patients with ovarian endometriosis, peritoneal endometriosis, and endometrial endometriosis. The mean ages of the control and study groups were 45.0 ± 6.56 and 40.9 ± 8.12, respectively. There was no statistically significant differences between the study and the control groups for age ($p = 0.073$).

The control group was compared with all study groups: endometrium of patients with endometriosis, and ovarian and peritoneal (incision) endometriosis groups. The absence or presence and intensity of nectin staining were compared. The presence of nectin staining and its intensity were higher in the study groups than those of the control group (Table 1).

The cases with endometriosis were compared with each other. No statistically significant differences were found between ovarian endometriosis and endometrium of patients with endometriosis ($p = 0.793$). No statistically significant differences were found between ovarian endometriosis and peritoneal endometriosis ($p = 0.494$). No statistically significant differences were found between endometrium of patients with endometriosis and peritoneal endometriosis ($p = 0.714$) (Kruskal Wallis test).

Nectin-4 expression was not detected in 25% (n=5) of the control group (Figure 1). Weak and moderate expression was 45% (n=9) and 30% (n=6) (Figures 2, 3). Nectin-4 was not

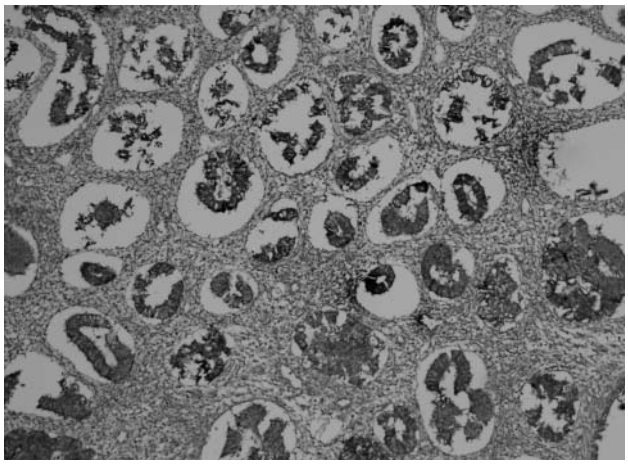

Figure 4. — Strong positive expression is observed for Nectin-4 in the endometrium of patients with endometriosis (immunostaining x200).

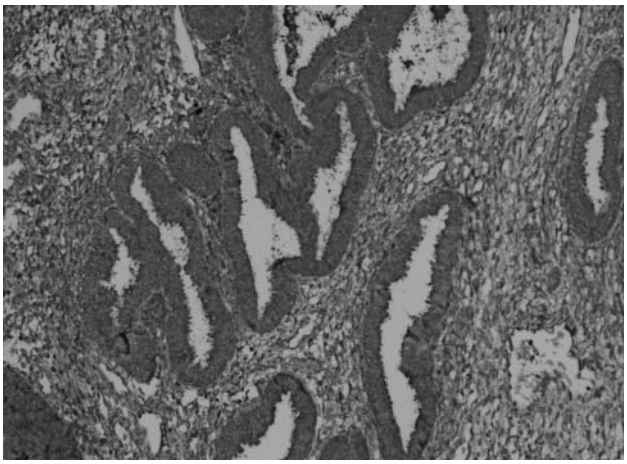

Figure 5. — Strong positive expression is observed for Nectin-4 in the ovarian endometriotic tissues (immunostaining x200).

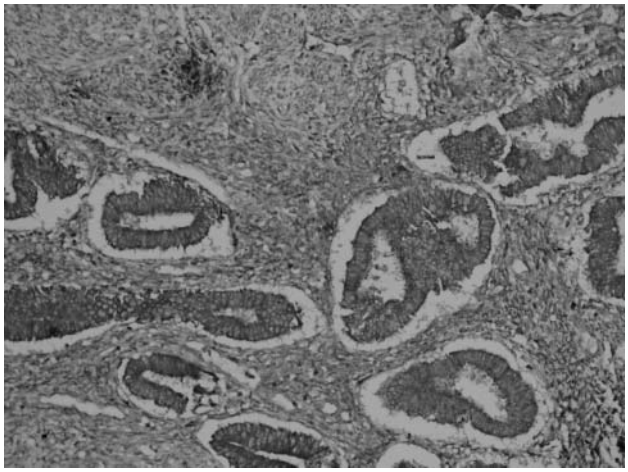

Figure 6. — Strong positive expression is observed for Nectin-4 in the peritoneal endometriotic tissues (immunostaining x200).

detectable in 5% (n=1) of endometrium of patients with endometriosis. Weak, moderate, and strong expressions were observed in, respectively, 20% (n=4), 45% (n=9), and 30% (n=6) of these cases (Figure 4). Weak, moderate, and strong expressions of Nectin-4 were observed in, respectively, 20% (n=4), 50% (n=10), and 30% (n=6) of ovarian endometriosis cases (Figure 5). In peritoneal endometriosis cases, weak, moderate, and strong expressions of Nectin-4 were observed in 35% (n=5), 55% (n=11), and 20% (n=4) of the patients, respectively (Figure 6). Nectin-4 expression was observed in all of the ovarian and peritoneal cases.

Discussion

Endometriosis is a benign disease characterized with development of endometrial tissues outside of the uterus. It leads to pelvic pain and infertility. The accurate diagnosis of the disease can be made via detailed gynecologic history and careful clinical examination. For its treatment, early accurate diagnosis is required.. Not all cases are suitable for medical treatment. Surgical treatment may be recommended, but morbidity is proportional to the severity of the lesion. For ectopic implantation of endometrial cells, a complex interaction between host tissue and epithelial endometrial cells is needed. Estrogen-induced endometrial cell growth, induction of angiogenesis, and lymph-angiogenesis play role in the development of endometriosis [8].

Nectins and nectin-like molecules (Necls) have been recently defined as immunoglobulin-like cell adhesion molecules, creating tight junctions between the cells. and regulating the adherence junctions [9]. Nectins are also involved in cellular polarization, differentiation, movement, proliferation, and survival [10]. Furthermore, some studies have established that nectins/Necls take part in tumorigenesis, with participation of Necl-2 in cellular proliferation, Nectin-4 in metastasis, and Nectin-3 in apoptosis [9]. Nectin-1 also plays important role in neurogenesis as axon guidance [11]. Moreover, it also plays important role in the development of endometriotic nodules and small nerve fibers responsible for endometriosis-related pain [12]. For these reasons, previous studies implicated these pathways in the pathogeneses of endometriotic lesions [8,13].

In a couple of previous studies on Nectin-4, it was reported that Nectin-4 expression might be an indicator of poor prognosis for malignancy. In the study of Athanassiadou *et al.* [6], it was reported that increased expression of Nectine-4 in breast tumors shows correlation with increasing tumor grade, lymph node metastasis, tumor size, and decreasing survival. As a result of this study, it was stated that the increasing Nectin-4 expression may be an indicator of poor prognosis in patients having breast cancer. In the study of Derycke *et al.* [14], it was suggested that the overexpression of Nectin-4 in cancerous tissues and the increase in Nectin-4 level in serum with

CA 125 may be useful in distinguishing ovarian cancers from benign gynecological diseases. In the study of Takano *et al.* [15], it was determined that Nectin-4 showed high expression in immunohistochemical staining in non-small-cell lung cancers, and that it is correlated with poor prognosis. At the end of the study, they stated that it may play important role in carcinogenesis of lung cancers, and that the serum and tissue biomarkers of this molecule may be a new candidate for therapeutic targeting. The main pathophysiological mechanism that has been put forward is that in contact with a suitable site, the Nectin-4 on the surface of ectopic or metastatic cells undergo cleavage, leading to increased intercellular adhesion and invasion of the site. This hypothesis may explain the metastatic potential of the endometrial cells. In addition, the invasive capacity of ectopic endometrial cells has been reported in previous studies by demonstrating the loss of E-cadherin expression [13, 16]. Finally, matrix metalloproteinases (MMP) and their natural inhibitors and the tissue inhibitor metalloproteinases (TIMPs) are involved in the invasive phase. Overexpression of MMPs and lower expression of TIMPs in the eutopic and ectopic endometrium and peritoneal fluid have been demonstrated [13]. It seems that Nectin-4 may participate in of the invasion of the subperitoneal space by ectopic endometrial cells in a way similar to E-cadherin and MMPs. In the study of Ballaster et al. [17], they investigated the contribution of Nectin-1, Nectin-3, Nectin-4, and Necl-2 to the pathogenesis of endometriosis. In the study, the staining densities of Nectins and Necl-2 were immunohistochemically compared in patients with peritoneal, ovarian, and colorectal endometriosis, and with endometriosis to negative controls. It was observed that the expression of Necl-2 was higher in all of the endometriotic lesions and endometrium of endometriosis patients than in the control group. Expression of Nectin-3 was found to be higher in the endometrium of endometriosis patients than the control group and all the endometriotic lesions and endometrium of endometriosis patients. Significant increase in Nectin-1 staining has been observed in colorectal endometriosis patients when compared to other endometrioses in other localizations. Nectin-4 staining has been shown to be stronger in the endometrium of endometriosis patients in proportion to endometrium of control group. No significant difference has been detected between the localizations of endometriotic lesions and Nectin-4 expression. In the present study, the expression of Nectin-4 was higher in endometriotic lesions in proportion to the endometrium of the control group. Moreover, Nectin-4 expression is higher in the endometrium of endometriosis patients than the endometrium of the control group.No significant difference was detected for Nectin-4 expression between the localizations of endometriotic lesion and the endometrium of endometriosis patients. As a result, the Nectin-4 expression has been detected to be significant in endometriotic lesions and endometrium of endometriosis patients. For this reason, the present authors believe that Nectin-4 may contribute to the pathogenesis of endometriosis, and thus it may serve as a target for the development of therapeutics.

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Address reprint requests to:
R. BEDIR, M.D.
Recep Tayyip Erdogan University
Faculty of Medicine, Department of Pathology
Islampasa Mahallesi, 53100, Rize (Turkey)
e-mail: bedirrecep@gmail.com