Cesarean Section Scar Endometriomas: Immunohistochemical Staining of Estrogen Receptor-alpha, and CD34

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Abstract

Objective: Cesarean section scar endometriomas (CSSEs) are believed to be the result of direct inoculation of the subcutaneous tissues or abdominal fascia with endometrial cells during surgery as well as higher immune tolerance during pregnancy. The ectopic endometrium exhibits multiple subtle, but biologically important, molecular abnormalities favoring increased production of estrogen, cytokines, prostaglandins, and metalloproteinases.

Patients and Methods: The present study was undertaken to immunohistochemically study the expression of the nuclear estrogen receptors (ERs) alpha and cytoplasmic endothelial cell markers CD34 in 34 cases of CSSEs, 27 cases of ovarian endometriomas (OEs) compared with 18 cases with late proliferative endometrium (PE) as control group

Results: The incidence of CSSEs is 0.39% in Mansoura university hospitals, the mean total score (TS) of ERs-alpha and CD34 assessed by mean vascular density (CD-34 MVD) were significantly increased ($p<0.001$) in cases of CSSEs and OEs versus the control group. There was a statistically significant increase in the TS of ERs-alpha ($p<0.001$) in cases of OEs versus CSSEs. On the other hand, there was no statistically significant difference in CD-34 MVD between cases of CSSEs and OVs. In cases with CSSEs, there was no significant correlation (r 0.212, p 0.229) between TS of ERs-alpha and CD-34 MVD. No significant correlations were noted between either ERs or CD-34 MVD and age of the patients, parity, number of prior CSs, duration since the last CS(s) and size of CSSEs.

Conclusion: CSSEs are a multifactorial disease, both ERs alpha and CD34 may play a role in the pathogenesis and maintenance of endometriosis. Obstetricians should keep in mind measures to prevent transmission of endometrial cells during CS.

Key words: Cesarean section scar endometriomas, ovarian endometriomas, immunohistochemical staining, CD34, ERs-alpha.

Introduction

Robert Mayer in 1903 was the first, who described the presence of endometriosis in the postoperative scar (1). Cesarean section scar endometriomas (CSSEs) was believed to be the result of direct inoculation of the abdominal fascia or subcutaneous tissues with endometrial cells during surgery (2). Other proposed theories are: higher immune tolerance during pregnancy and autoantibody formation (3,4). Circulating blood cells originating from bone marrow can differentiate into endometriotic tissues at various sites (5).

Although endometriosis is a nonmalignant disorder, the ectopic endometrium has the capacity to adhere, attach, and implant. It exhibits multiple subtle, but biologically important, molecular abnormalities, including the activation of oncogenic pathways or biosynthetic cascades favoring increased production of estrogen, cytokines, prostaglandins, and metalloproteinases(6).

Estradiol enhances the survival or persistence of endometriotic tissues. Moreover, it aggravates the pathological processes (e.g., inflammation and growth) and the symptoms (e.g., pain) associated with endometriosis. The predominant expression of ERs-alpha may be essential for the development and growth of peritoneal and ovarian endometriosis (7). There are scarce informations about ERs alpha in cases of CSSEs. Direct and indirect evidences have suggested that angiogenesis is a prerequisite for the development of endometriosis, and activation of angiogenesis for adequate blood supply is essential for the survival of the normal as well as ectopic endometrium (8-10)

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CD34, a sialomucin-type glycophosphoprotein, has potentially important roles in blood vessel formation in both embryos and adults. Under all circumstances, CD34 have been shown to facilitate cell migration, it seems to act as a signaling molecule. In endometriosis, it appears that, up to 37% of the micro vascular endothelium of the ectopic endometrial tissue originates from endothelial progenitor cells (11). CD34 were elevated in cases of endometrial carcinoma, and endometrial hyperplasia, than benign endometrium (12), yet it has not been studied before in cases of OEs and CSSEs.

The present study was undertaken to study the expression of ERs-alpha and CD34 in tissue biopsies from CSSEs and ovarian endometriomas (OEs) compared with late proliferative endometrium as control.

Subject & Methods

In the present study, 61 formalin-fixed, paraffin-embedded tissue biopsies obtained from Egyptian women during the period from June 2007 till May 2012, were immunohistochemically studied. Of these, 34 cases were CSSEs their data were retrieved from the archival materials of department of pathology and department of gynecology, Mansoura university hospitals. The clinico-pathological parameters (e.g. age, parity, symptoms as cyclic or non cyclic pain, size of endometriomas, number of prior CSs and the time interval between CS and the surgical excision) were retrieved from the hospital patient files, as well as the histopathological reports. It was not possible to check CS status whether it was selective or elective at delivery. Also, the presence of associated pelvic endometriosis in cases of CSSEs was not possible to assess.

The other 27 ones were patients with OEs underwent ovarian cystectomy or oophorectomy via either laparotomy or laparoscopy at Mansoura university hospitals. The control group included 18 age-matched cases with late proliferative endometrium, they have regular menstrual cycles and had not received any hormone therapy in the prior 6 months.

Exclusion criteria included: Patients with previous uterine surgery (e.g. myomectomy, hysterotomy, ectopic pregnancy, and previous tubal surgery), other abdominal operations as appendectomy, other extra-pelvic sites for endometriomas (e.g. episiotomy scar), and prior hormonal therapy 3-6 months before surgery and recurrent endometrioic lesions. Mansoura ethical committee approved the study.

Immuno-histochemical staining

From each paraffin block, 3 sections each of 4 μm-thicknesses were taken. One section was stained with Hematoxylin and Eosin for revision of the histopathological diagnosis; the criterion for diagnosis of endometrioma was the presence of endometrial glands and/or stromal cells in the tissues. The other 2 sections were dewaxed using xylol for 15 min, rehydrated in an alcohol-raw, and subjected to antigen retrieval on a high setting for 10 min in a pressure cooker in sodium citrate buffer (pH 6.0) containing citric acid 0.1 M and sodium citrate 0.1 M in distillate aqua. After cooling, the slides were washed twice in phosphate buffer solution (PBS). Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide (Merck) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with "diluted normal serum" for 20 min at room temperature. Sections were evaluated by two expert pathologists, who were blinded about the clinical data of patients. Results from both were collected and the mean of the results was used.

ERs-alpha was diluted in diluting medium (Dako, Glostrup, Denmark) for a further 30 min and repeated washing with PBS, visualization was performed with DAB for 8-10 min. The slides were further counterstained with Mayer's acidic Hematoxylin and washed in an alcohol-raw (50-98%). Negative controls were performed by replacing the primary antibody with normal mouse serum. Positive cells showed a brownish nuclear reaction (fig 1A, 2A), ERs-alpha was evaluated by the total score (TS) (13). The TS is the sum of intensity score (IS) and proportion score (PS). IS was graded as 0 point = no staining reaction, 1 point = weak staining, 2 points = moderate and 3 = strong and PS was graded as 0 point = cell nuclei completely negative, 1 point = 1% positive nuclei, 2 points = >1-10% positive nuclei, 3 points = >10 -33% positive nuclei, 4 points = >33-66% positive nuclei, 5 points = > 66-100% positive nuclei.

CD34 Primary CD34 antibodies were prediluted. The slides were then incubated for 1 hour at 37°C and kept at 4°C in a humid chamber, after washing the sections with PBS, biotinylated anti-mouse IgG was applied to slides, followed by incubation and rinsing with a stream of PBS. Conjugated antibodies were visualized with Diaminobenzidine (DAB) chromogen stain. Sections were counterstained with Mayer's Hematoxylin for 1–2 minutes, dehydrated and mounted. For each case, a negative control was applied by replacing the antibody by PBS or nonimmune serum. Brown cytoplasmic staining of CD34+cells was considered positive reaction (fig 1B, 2B). Each brown stained cell or cell cluster that was clearly separated from adjacent microvessels, endometrial cells and other connective tissue elements were considered as a single countable microvessel. Initially, the most vascularised tumor areas containing the greatest number of capillaries and small venules (so called neovascular hot spots) were selected under low power (x40 and x100) using a light microscope. Five hot spots were taken, high-power (x400) fields were then chosen randomly, and the number of microvessels in each high power field was counted in each sample (14). Vessels characterized by thick muscular walls or with lumen greater than 20μm in diameter were excluded from the count. Mean vascular count (MVC) was calculated as the mean of the 5 values obtained. CD-34 MVD was calculated by dividing the MVC of the examined fields on the high-power field area which is 0.74 mm2.

Statistical analysis was carried out via Statistical package for social Science (SPSS) version 17 program on windows XP. Qualitative data were represented in the form of number and frequency, while quantitative data were represented in the form of mean ± standard deviation (mean±SD). Kolmogrov-smirnov test was used to test normality of quantitative data. Student's t test was used to compare groups. Whereas, Pearson's correlation test was used to determine correlation between variables. Results were considered significant if p value less than or equal 0.05.

Results

During the study period, 37 cases with histologically confirmed abdominal wall endometriomas, 3 cases were excluded: one case following ectopic pregnancy, and one case following abdominal hysterectomy, and one was recurrent lesion and 34 cases were diagnosed with CSSEs, among 10,136 women underwent CS, giving an incidence of CSSEs about 0.34% (table 1). The mean age of the patients at the time of surgical excision in CSSEs was (28.8 + 6.09) years range (22-42), in patients with OEs, it was (29.11 + 5.19) years range (20 – 41), while in women with PE, it was (29.72+7.31) years range (18-44) years. There was no statistically significant difference between all groups.

In CSSEs, the mean number of parity was (2.56 ± 1.11), with a range from 1 to 6, the mean number of prior CSs was (2.12 ± 0.91), with a range from one to four, 25 patients (61%), had one prior CS. All patients complained of scar nodule(s), cyclic pain in the affected area, relating to the menstrual period was present in 23 patients (67.7%). Non cyclic pain in the scar area, without any relationship with the men¬strual period, was reported by 11 patients (32.3%). The mean duration of between CS(s) and surgical excision was (30.88± 12.07) with a range from (9-58) months.

The mean size of CSSEs, defined the largest single diameter, (5.36 ± 1.39) ranged from (2.5-6.7) cm. Histopathologically in 30 cases (91%), the endometrial glands and stroma were within in a background of fibro adipose tissue (subcutaneous), while in 3 cases skeletal muscle fibers were present (subaponeurotic) (table 1).

ERs-alpha were expressed in all tissue biopsies both in the endometrial glandular and stromal cells in all groups. The mean TS of ER-alpha was increased significantly ($p<0.001$) from the OEs (5.52 + 1.40), than CSSEs (4.53 + 1.26), versus the control group (2.85+ 1.38). Interestingly, there was a statistically significant increase ($p <0.005$) among cases of OEs versus CSSEs (table2). The mean CD-34 MVD was increased significantly ($p <0.001$) in cases with CSSEs (45.50+ 19.67) than control group (28.50+13.81). Also, it was increased significantly ($p <0.001$) in cases with OEs (41.30+17.57) than control group. However, there was no statistically significant difference ($p <0.44$) between cases of CSSEs and OEs (table 2).

In cases with CSSEs, there was no significant correlation ($r = 0.212$, $p =0.229$) between TS of ERs-alpha and CD-34 MVD. Also, no significant correlations were noted between either TS of ERs-alpha or CD-34 MVD, age of the patients, size of CSSEs, number of prior CS(s) (table 3). There was no statistically significant change between either TS of ERs-alpha or CD-34 MVD in relation the type of pain or size of CSSEs (data not shown). In CSSEs, scar lesion(s) were excised > 20 months in 28 patients, and in 6 patients < 20 months after prior CS(s), there was a significant increase ($P < 0.05$) in both CD-34 MVD and TS of ERs-alpha and duration > 20 months (table 4).

Discussion

The incidence of CSSEs in the present study was 0.34%, Nominato et al (15) reported the incidence of scar endometrioma in 0.2% women submitted former to CS, others reported an incidence of 0.29%, and a relative risk of 27.3 for the occurrence of surgical scar endometriosis following CS(4). Our results were higher probably due to different selection criteria as we selected only cases with CSSEs. The average age of the patients in the present study was 28 years, it was younger than that reported by (16), this may be due to tendency toward earlier marriages and consequently earlier deliveries in our patients. We did not find any correlation between the number of parity and the occurrence of CSSEs, these results were in agreement with (16). On contrary low parity may increase the risk of CSSEs (17). The number of prior CSs did not increase the incidence of CSSEs, these were in agreement with (17, 18). In 23 patients (67.7%) cyclic pain was present, this was concordant with others (16, 19).

ERs alpha in CSSEs and OVs ERs-alpha were immunohistochemically expressed both in the glandular and stromal cells in all studied groups. The mean TS of ER-alpha was increased significantly ($p<0.001$) in the cases of OEs, than control group. These results were in agreement with others (7,17). Also, it was increased significantly ($p<0.001$) in the cases of CSSEs, than control group. Our study was among the first ones to address ERs-alpha in CSSEs. Interestingly, there was a statistically significant increase ($p <0.001$) among cases of OEs versus CSSEs. The exact causes of these findings were unknown it may indicate that estradiol may be more important in the pathogenesis OVs rather than CSSEs, other factors may contribute to the development of CSSEs. Also, there was a lack of literature data confirming relation of hyperestrogenemia to scar endometriomas. These findings highlight the importance of estradiol both in the pelvic and CSSEs. It is well known estradiol enhances the survival or persistence of endometriotic tissues, it aggravates the pathological processes (e.g., inflammation and growth) and the symptoms (e.g., pain) associated with endometriosis. Moreover, ERs are ligand-dependent transcriptional factors, which can bind to different DNA sites to initiate the expression of specific genes. In addition, indirect mechanisms through contacts with DNA-bound transcription factors have reported (7).

CD-34 MVD in CSSEs and OVs Vascular density was quite heterogeneous in any given tissue sections of the various histological types, some areas being vascular and other areas remaining relatively avascular. The mean CD-34 MVD was increased significantly ($p<0.001$) in cases with OEs than control group. In vitro study confirmed these results in mice (20). The mean CD-34 MVD was increased significantly ($p<0.001$) in cases with CSSEs than control group. There was no statistically significant difference ($p >0.05$) between cases of CSSEs and OEs. The present study is among the first ones that study the expression of CD 34 in CSSEs. The potential causes of increase in CD34 in both OEs and CSSEs may be due to chronic overstimulation of endothelial cells leading to cell activation and proliferation leading to angiogenesis. Regardless of its possible causes, angiogenesis is of paramount importance in the growth and survival of endometriotic lesions as ectopic lesions require nutritional supply to maintain proliferation and to invade into ectopic sites within the host. Thus, CD34 may appear to be part of the complex interplay of sialomucins contributing to the maintenance of endometriosis.

The potential significance of angiogenesis, it may allow for the identification of patients at high risk of recurrence after surgical excision who may benefit from aggressive surgical procedures as well as postoperative therapy. Also, the potential antiangiogenetic therapy as a method of treatment of endometriosis. In cases with CSSEs, there was no significant correlation between TS of ERs-alpha and CD-34 MVD. This highlight the concept that both pelvic and CSSEs are a multifactorial disease. Also, there was no significant correlations between either of CD-34 MVD nor ERs-alpha and the size of CSSEs. Increased expression of both and CD-34 MVD and ERs-alpha in tissue biopsies of CSSEs more than 20 months after prior CS, may indicate that both factors are important both in the pathogenesis and progression of OEs and CSSEs and may explain the aggressiveness nature of endometriosis.

Since the results of the present study supports the theory of iatrogenic cell transportation, as well as in the face of increasing rates of cesarean deliveries, obstetricians should adopt measures to prevent CSSEs, first of all unnecessary CS(s) should be avoided and, if CS is performed careful surgical techniques as (1) Perform selective CSs after the onset of spontaneous labor, whenever possible, instead of elective CSs as the onset of labor marks the termination of pregnancy- induced immune tolerance to the implanted endometrial cells. Wicherek et al.,(4) stated that performing CS(s) without the presence of labor conditions more than doubles the risk in relation to situations in which cervical ripening and uterine contractions are present. (2) Shielding the wound by a quadrangular bandage during placental extraction and during curettage of the uterine cavity and immediately discard swabs or sponges used for cleaning the uterine cavity (21). (3) Avoid penetration of the endometrium during suturing the myometrium as reported by (22). (4) Failure to close the parietal and visceral peritoneum in the CS may be related to greater rates of CSSEs (23), although an

evidence based obstetrics recommend during cesarean section to leave the visceral peritoneum unsutured. We advise multicenter randomized control to substantiate or refute these steps. (4) Thorough washing the abdominal wall via irrigation with a salt solution before definitive closure (24). (5) Also, it is recommended not to use the same surgical material and the same in¬struments as used in hysterorraphy, when suturing other abdominal wall layers as stated by(25). (6) Ongoing use of high doses of progesterone during the first six months after CSs in order to decrease the occurrence of endometriosis at the surgical site (26). (7) Prolonged breast feeding is well known protecting factor because of causing hypoestrogenic status that does not support endometriosis development (26). Although, there are no randomized controlled trials that can support these maneuvers, we advise adopting these steps during the surgical procedures.

We can conclude that CSSEs is a multifactorial disease, both ERs alpha and CD34 may play a role in its pathogenesis and maintenance. Obstetrician should keep in mind measures to prevent transmission of endometrial cells during CS.

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