A CLINICO-PATHOLOGICAL STUDY ON ENDOMETRIOSIS WITH SPECIAL REFERENCE TO THE CD10 MARKER

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ABSTRACT

BACKGROUND

Endometriosis is defined as the presence of ectopic endometrial tissue outside the uterine cavity and is characterized by recurrent episodes of pelvic pain and dysmenorrhea. The high prevalence of the disease along with its recurrent persistent nature poses a significant burden on the healthcare system of a country. Considering the enormity of the healthcare burden and the impact it has on the quality of life of the patients, here was a need to develop a marker which may help in the definitive diagnosis of this dreadful disease. The aim of this study is to corroborate the histopathological diagnosis of endometriosis with the immunohistochemical staining for CD10 and to find out the diagnostic efficacy of CD10 for endometriosis among clinically diagnosed patients of endometriosis.

METHODS

Patients admitted with suspected endometriosis and planned for operation in the Department of Gynaecology and Obstetrics, at North Bengal Medical College and Hospital in that year who gave informed consent for the study were included in the study provided they satisfied the inclusion and exclusion criteria. A representative histological block from each of the biopsy specimen harvested during the surgery of patients of endometriosis was chosen for immunohistochemistry for CD10. Correlation of CD-10 antigen expression was done with the post-operative histopathological findings.

RESULTS

Immunohistochemistry with CD10 increased the diagnostic yield of endometriosis cases. Cases diagnosed as endometriosis by routine histopathology were found to be positive for CD10. In others, CD10 positivity was also seen in those characterised by haemorrhagic cysts and endometriotic stroma.

CONCLUSIONS

CD10 immunostaining can be used to give a definitive diagnosis in cases where previously the pathologist would report as being "consistent with endometriosis" even in the absence of endometrial epithelium. We strongly recommend the use of CD10 IHC to confirm or exclude the diagnosis in cases of presumptive endometriosis and in those mistaken for this entity.

KEY WORDS

Endometriosis, Histopathological Diagnosis, Immunohistochemical Staining for CD10, CD10 Positivity

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BACKGROUND

Endometriosis is defined as the presence of ectopic, locally invasive, functional, non-neoplastic endometrial tissue (glands and stroma) outside the uterine cavity. A chronic persistent disease characterized by recurrent episodes of pelvic pain, dysmenorrhea and unexplained infertility, endometriosis has a prevalence of 10% among the premenopausal women all over the world, of whom approximately 30–50% are symptomatic.[1] In India, the prevalence of the disease is estimated to be as high as 54.98 %.[2]

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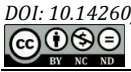

The risk of endometriosis among Orientals appears to be higher than whites. The high prevalence of the disease along with its recurrent persistent nature poses a significant burden on the healthcare system of a country. Since the highest prevalence of the disease is during the 35-44 years, endometriosis also heavily affects the quality of life of diseased women all through their reproductive years. Therefore, the disease demands professional attention, especially when fertility is impaired or chronic pelvic pain affects the lifestyle and the psychological status of the patient.

No single symptom is pathognomonic of endometriosis and there exists no confirmatory blood tests for diagnosis of endometriosis. A blood test with a high sensitivity would be useful to identify women with symptomatic endometriosis but not detectable by ultrasound imaging. During menstruation, an increased level of CA-125 has been shown in women with and without endometriosis.[3] But the increase is more pronounced in women with endometriosis. Though some studies have advocated the use of ratio of CA-125 (Menstrual versus follicular values >1.5) for diagnostic purposes, attempts have been limited by low sensitivity of CA-125 (20%-50%).[4] Considering the enormity of the

healthcare burden and the impact it has on the quality of life of the patients, there was a need to develop a marker which may help in the definitive diagnosis of this dreadful disease. CD10 or common acute lymphoblastic leukaemia antigen (CALLA) is a useful immunohistochemical marker expressed by haematopoietic neoplasms such as acute lymphoblastic leukaemia and follicular lymphomas and also some non–haematopoietic tissues like normal endometrial stromal cells. Recent studies have shown that immunohistochemical staining with CD10 may be useful in identifying endometrial stromal cells at ectopic sites. The aim of this study is to corroborate the histopathological diagnosis of endometriosis with the immunohistochemical staining for CD10 and to find out the diagnostic efficacy of CD10 for endometriosis among clinically diagnosed patients of endometriosis.

METHODS

This hospital based cross-sectional descriptive study was conducted in the Department of Pathology in association with the Department of Gynaecology and Obstetrics, at North Bengal Medical College and Hospital over a period of one year. All patients in the age group 16-50 years admitted with suspected endometriosis and planned for operation in the Department of Gynaecology and Obstetrics, at North Bengal Medical College and Hospital in that year and gave informed consent for the study were included in the study. Any patient with a known history of endometrial carcinoma, endometrial stromal sarcoma, or lymphoma where the CD-10 positivity is known were excluded from the study. All the relevant history pertaining to age, parity, chief complaints and duration of complaints were collected. After detailed clinical examination which included local examination, per-abdominal, per-speculum and bi-manual examination and pre-operative investigations like ultrasonography, the patient underwent the advised surgery. The data were entered in the pre-designed, pre-tested proforma.

The biopsy specimen harvested during the surgery was sent to the pathology laboratory where it was first measured as to its size and weight and then labelled by unique serial numbers. The whole specimen was sliced and sent for processing. Evidence of haemorrhage was noted in the myometrium and ovary. The processing of the tissue included dehydration by increasing strengths of alcohol for 1-3 hrs., then clearing by xylene for 1-3 hrs., followed by impregnation in molten paraffin for 5 hrs., in a hot air oven. The tissue was embedded in molten wax in Leukart's L blocks properly oriented to enable cutting along with complete identification. Several sections of 3-5 microns thickness were cut out with the help of a microtome. These sections were then teased onto and fixed onto slides coated with egg albumin and then dried for 30 mins. The slides were then deparaffinized by dipping into xylene and stained first by haematoxylin and then eosin after hydration and dehydration by graded concentrations of alcohol. Finally, the sections were mounted by coverslip and observed under microscope to observe the endometrial glands, stroma, haemorrhagic areas, and hemosiderin laden macrophages.

A representative histological block from each of the cases was chosen for immunohistochemistry. Sections were taken on Poly-L-Lysine coated slides and air-dried for 30 mins., at room temperature followed by quick baking in a hot-air oven at 60 degree Celsius for 30 mins. The slides were dewaxed and rehydrated with graded alcohol and antigen retrieval was accomplished by boiling the slides in TRIS-EDTA buffer (pH=9.0) in a pressure cooker. After washing, peroxide free blocks were placed on the sections and incubated for 10 mins in humid chamber. Following this primary antibody (Antibody to CD 10) was added and the slides were incubated for 60 mins at room temperature. Secondary antibody in the form of horse-radish peroxidase was added on the slides after the requisite washing and then incubated for 30 mins in a humid chamber. The slides were stained with diaminobenzene (Chromogen) and counterstained with haematoxylin. The slides were then washed, dehydrated with graded alcohol, air dried and finally mounted for observation. Final interpretation was done using Olympus CH 20i microscope. The following parameters were studied to assess the expression of CD-10 antigen by the IHC -

a. Cytoplasmic and membrane positive endometrial stromal cells.
b. Intensity of staining.

Correlation of CD-10 antigen expression was done with the post-operative histopathological findings.

RESULTS

The mean age of presentation was 27.11 years with the maximum number of patients in the age-group 21-25 years (37.7%) followed by that within 26-30 years (33.3%).The chief complaints were dysmenorrhoea (26.7%), primary infertility (22.2%), vague lower abdominal pain (17.8%), pain and bleeding from scar mark (8.9%), secondary infertility and pain abdomen (2.2 %) among others. Most patients (64.44%) were nulliparous at presentation. Per-abdominal examination did not yield any positive findings in 71.1 % while others showed bi-lateral or unilateral abdominal lump, nodularity and or tenderness at scar mark etc. The classical finding of endometriosis i.e. blackish puckered spots was found in the fornix in only 12 cases (26.67%) on per-speculum examination. Bimanual examination yielded no positive findings in 51.1% of patient. In others, tender fixed retroverted uterus (11.1%) and nodules in POD (17.8%) were the main findings. Pre-operative imaging demonstrated bi-lateral tubo-ovarian mass with mixed echogenicity in 53.3% cases. Significant per-operative laparoscopic findings were bilateral ovarian cysts filled with chocolate coloured material with gross adhesion in 15.6%, ovarian cysts filled with chocolate coloured material without gross adhesion in 17.8% and chocolate coloured material at scar mark in 13.3%. Routine histopathology corroborated with the clinical diagnosis in 55.6% (25 cases) where endometriotic glands and stroma were found and haemorrhagic cysts were seen in 11.1% (5 cases). Immunohistochemistry with CD10 increased the diagnostic yield to 31 endometriosis cases. 25 cases diagnosed as endometriosis by routine histopathology were found to be positive for CD10. Out of the 5 cases diagnosed as haemorrhagic cysts, 2 were CD10 positive thus proving the presence of endometriotic stroma. 2 cases where only glands were obtained were all CD10 positive, supporting the earlier studies that endometriotic stroma remain intimately attached with glands though may not be appreciable by routine histopathology.

Histopathological Finding (H/E Stain)	No. of Cases	Diagnosis (H/E Stain)	CD10 Positive Cases
Endometrial glands, Endometrial stroma, macrophages	25	Endometriosis	25
Cysts, haemorrhage, macrophage	4	Haemorrhagic cysts consistent with endometriosis	2
Cysts, haemorrhage	5	Haemorrhagic cysts	2
Endometrial glands	2	Consistent with endometriosis	2
Follicular cyst	2	Follicular cyst	nil
Old hyalinized villi	1	Old ectopic pregnancy	nil
Multiple serous cysts	1	Serous cystadenoma	nil
Cysts lined by cells filled with mucin	2	Mucinous cystadenoma	nil
Haemorrhagic corpus luteum	3	Corpus luteal cyst	nil

Table 1. Showing Comparison of Histopathological Finding and Diagnosis by Routine H/E Stain with CD10 Immunohistochemistry Findings

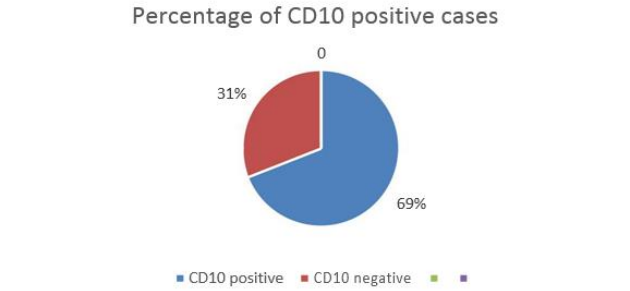

Figure 1. Pie-Chart Depicting the Percentage of Cases Which Were CD10 Positive

The number of cases diagnosed provisionally by histopathological findings and those by CD10 immunohistochemistry findings are briefly tabulated below. (Table 1). So, finally the total number of cases diagnosed as endometriosis by routine stain (25 out of 45; 56%) was increased (31 out of 45; 68.9%) after using CD10 immunohistochemistry. The percentage of patients diagnosed by CD10 immunohistochemistry is depicted pictorially in the pie-chart given below. (Figure 1)

DISCUSSION

Endometriosis is one of the most important benign gynaecological conditions, second only to the most common cause of surgical interventions, namely uterine fibroids, in women under 45 years. The present study was done to correlate the clinic-pathological diagnosis of endometriosis and corroborate the histopathological diagnosis with CD10 immunohistochemistry in 45 cases of endometriosis who underwent surgical treatment in the Department of Gynaecology & Obstetrics, NBMCH. After receiving the biopsy specimen of the patients (Those with a clinico-radiological suspicion of endometriosis) in the Department of Pathology, proper histopathological examination was done in Haematological Eosin stained sections followed by immunohistochemical staining of formalin fixed paraffin embedded sections for CD10 antigen. Only 25 cases out of 45 could be diagnosed as endometriosis following histopathology and laparoscopy. After Immunohistochemistry was performed, all the cases clinically diagnosed as endometriosis turned out to be positive for CD 10 antigen.

The cell surface metalloendopeptidase CD 10, (also known as enkephalinase or neprilysin) is a human 100-kd membrane-associated neutral endopeptidase, identical to the acute lymphoblastic lymphoma antigen.[5]It was first used in the diagnosis of ALL and later for other lymphoid and non-lymphoid malignancies. CD10 immunoreactivity has been used most commonly in the diagnosis of precursor B-cell leukemia, follicular lymphoma, and Burkitt lymphoma.[6] CD10 has also been demonstrated to be actively involved in the growth and differentiation of haematopoietic and epithelial cells and so has been detected in normal cells as well like the bile canalicular surface of hepatocytes and the brush border of normal small bowel enterocytes.[7-11] CD 10 also functions as a cell surface peptidase involved in the biologic activity of peptide hormones. Thus, CD10 may be expressed in many hormone-sensitive and peptide-sensitive cells and their corresponding neoplasms. In addition to this, CD10 is commonly expressed in myoepithelial cells of breast, renal tubular and glomerular cells, renal cell carcinoma, hepatocellular cancers, pulmonary alveoli, acute lymphoblastic leukaemia and lymphoma and normal and ectopic endometrial stromal neoplasms and adenomyosis and has been demonstrated to be a marker of endometrial stromal differentiation. Significantly, this CD10 positivity of endometrial stroma is maintained even when the tissues are discovered at ectopic locations outside the uterus. This property of CD10 positivity in endometrial stromal cells, was first reported by Imai et al[12] by the indirect immunofluorescence technique. After that several studies have demonstrated that the diagnostic yield may be increased by CD10 immunohistochemisty.[13,14,15] Now-a-days, a monoclonal antibody (Clone 56C6) is commercially available for paraffin immunohistochemistry thus helping further research on this issue.[16]

The diagnosis of endometriosis largely depends on laparoscopic optics and video monitors, but the appearance of the affected tissue often mimics endosalpingitis, cancer and pelvic infection. It is important to confirm the diagnosis of endometriosis as the disease is associated with a chronic course. As histology may lead to unexpected results, so an improved method of detection of endometriosis is necessary specially when the diagnosis is suspected but investigations are ambiguous or there is a morphological doubt. In long standing cases, diagnosis may be difficult due to fibrous obliteration and paucity of endometrial stroma. In some cases, it may be difficult to distinguish between non-specific fibrous stroma, ovarian or endometrial stroma. In some others when endometrial stroma may be present without the associated glands as in as stromal endometriosis. CD 10 is helpful to detect ovarian endometrial cyst where repeated haemorrhages show the cyst to be lined by several layers of hemosiderin-laden macrophages. Previously the pathologist would report these as being “consistent with endometriosis” but now using CD10 immunostaining a definitive diagnosis of endometriosis can be made even in the absence of endometrial epithelium. CD 10 immunohistochemistry has thus helped to detect endometriotic components at ectopic sites like the, fallopian tube or diaphragm.

In this study, addition of CD10 IHC improved the rate of histologic detection from 35% to 45% of lesions examined, resulting in a new diagnosis of endometriosis in three of twelve women with negative results on H/E staining. All the

three patients had minimal endometriosis as per the revised ASRM classification. But, there are several potential limitations to diagnose by CD10 IHC. Firstly, CD10 is not specific enough for endometrial stroma as it is also identifies lymphocytes. So, a specimen with lymphoid infiltration will be positive for CD10 and may be falsely considered to indicate an endometriotic lesion. Secondly, CD10 IHC will stain other non-endometriotic lesions like adenomyosis or mesenchymal tumors which are generally not confused with endometriosis on H/E staining. Therefore, the findings should be carefully interpreted in the context of the overall clinicopathologic picture to avoid confusion or misdiagnosis.

CONCLUSIONS

CD10 IHC may help in the detection of ectopic endometrial stroma or distinguishing endometriosis from other confusing entities. We strongly recommend the use of CD10 IHC to confirm or exclude the diagnosis in cases of presumptive endometriosis and in lesions that may be mistaken for this entity.

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