Use of CD138 as Diagnostic Tool for Chronic Endometritis in Infertility

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Abstract

Background: Chronic endometritis (CE) can present in different ways. Various studies have proved the association between CE and infertility. As the data on the analysis of CD138 immunostain as a diagnostic tool for CE is scarce, this study was designed to compare CD138 with H&E for the diagnosis of CE.

Objective: To compare the results of CD138 immunostain with Hematoxylin and Eosin staining for diagnosis of chronic endometritis.

Methodology: Study design: Cross-sectional study. Place of study: Shifa International Hospital, Islamabad. Duration of study: January to September 2018. A total of 100 patients meeting selection criteria were included in the study. All patients had at least one-year history of infertility. Specimen were assessed on both H&E and CD138 immunostain and then results were compared. Data were analyzed by using SPSS version 20.

Results: A total of 100 biopsies from females having infertility were included in the study. The mean age of patients was 33.4±4.3 years. A total of 32 out of 100 cases were found to be positive on H&E stain and 36 cases were positive on CD138 and 27 cases were true positive and 59 were true negative.

Conclusion: CD138 has increased sensitivity in diagnosing Chronic Endometritis as comparing it with conventional H&E staining.

Keywords: Abnormal Uterine Bleeding, Chronic Endometritis, Infertility, Plasma Cell, Syndecan-1, CD138, H&E staining.

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Introduction

Chronic Endometritis (CE) is defined as ongoing inflammation of the endometrium and can present in different ways.[1] The patients are usually asymptomatic or may have subtle abnormalities such as abnormal uterine bleeding, pelvic pain, dyspareunia, leucorrhea, and/or infertility. Various studies have proved the association between CE and infertility.[1] CE is identified in 30% of patients with repeated implantation failure, 28% of the patients with unexplained infertility, and 12% of patients with unexplained recurrent miscarriages.

Diagnosis of CE is of utmost importance and is difficult to achieve but lacks international consensus. Many diagnostic modalities are present like histology, microbiological cultures, hysteroscopy, and molecular microbiology. Despite all these diagnostic methods endometrial biopsy is considered the gold standard and the most advantageous approach so far. Histopathological diagnosis requires an increased number of plasma cells (PCs) in endometrial stroma.[4]

Conventional Haematoxylin and Eosin (H&E) staining is not too supportive in the identification of true plasma cells in a busy endometrial stroma. PCs have many mimickers that may be masked due to dense mononuclear infiltrate, increased plasmacytoid stromal cells, abundant stromal mitosis, pronounced predecidual reaction, menstrual features, or secondary changes due to exogenous progesterone treatment prior to biopsy.[5] True PCs have a characteristic morphological appearance which shows clock face chromatin, eccentric nucleus, and a visible peri-nuclear halo. For the past few years, much work has been done to introduce and standardize the supplementary diagnostic techniques in the identification of PCs. Certain techniques include methyl green pyronin staining, immunohistochemistry (IHC) for immunoglobulin G, syndecan-1 protein (CD138), and in-situ hybridization for κ and λ light chains. Syndecan-1 is a cell surface proteoglycan that is strongly expressed on PCs but not on other cells like lymphocytes, eosinophils, neutrophils, histiocytes, or endometrial stromal cells.[6]

As the data on the analysis of CD138 immunostain as a diagnostic tool for Chronic Endometritis is scarce, this study was designed to compare CD138 with H&E for diagnosis of CE; so that in the future we may adopt CD138 as a diagnostic tool for

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endometritis (chronic) in our local population.

Methodology

A cross-sectional study was conducted in the Department of Histopathology, Shifa International Hospital, Islamabad. Approval was taken from the ethical review committee of Shifa International Hospital. A total of 100 consecutive patients meeting selection criteria were included in the study. These patients reported between January to September 2018. Samples of female patients with infertility were included in this study. Most of the specimens were received from Medicsi Center, Islamabad, which is one of the leading units for the treatment of infertility in Pakistan. These samples were received and processed at the Pathology Department of Shifa International Hospitals, Islamabad, and assessed by using both H&E stain and IHC for CD138 antibody. For hematoxylin and eosin staining the blocks were cut at 5 micrometers. Two slides were prepared and each slide contained two levels. Almost 15 min on average were spent for examination of each H&E slide on light microscopy. Formalin-fixed, routinely processed and paraffin-embedded tissues were cut at 4 micrometers and then stained with mouse monoclonal antibody CD138/syndecan-1 (B-A38) on VENTANA detection kit and accessories. Slides were then baked for at least 2 hours in the oven at 60°C. Slides were then counterstained with hematoxylin, dehydrated, mounted, and examined. Samples which were inadequate, non-representative, and/or autolyzed were excluded from the study.

The number of plasma cells was blindly calculated on both H&E and CD138 immunostain. Weak positivity of glandular epithelial cells worked as an internal positive control. Those PCs having strong membranous circumferential staining of CD138 were taken as positive. The dot like and weak staining was considered negative. PCs were calculated in 10 high power fields. CE was then graded as follows. <5 PC/10HPF (insignificant for CE), 5-15 PC/10HPF (mild CE), 16-25 PC/10HPF (moderate CE) and >25 PC/10HPF (severe CE). Data were analyzed with Statistical Package for Social Sciences (SPSS) version 20.0. Quantitative variables like age were presented in terms of mean ± SD (Standard Deviation). Frequency was calculated for qualitative variables (TP, TN) such as presence or absence of Chronic Endometritis on CD138 and H&E. Effect modifiers like age was controlled through stratification. Post-stratification chi-square test was applied. P-value ≤0.05 was considered statistically significant.

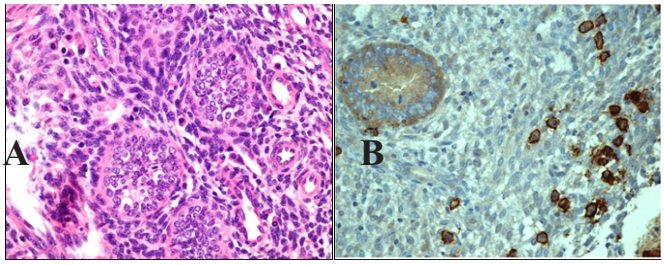
Results

A total of 100 biopsies from females having infertility were included in the study. The mean age of patients was 33.4±4.3 years with minimum and maximum age as 20 and 44 years respectively. Positive endometritis on H&E was found in 32% of cases while 36% cases were diagnosed to have positive endometritis on CD138 (Figure-I A&B). Whereas 5 cases showed positivity on H&E but were not confirmed on CD138 immunostain, and 27% cases were found to be positive on both H&E and CD138 staining (true positive) while 59% cases were negative on both diagnostic modalities (true negative). The p-value was found to be statistically significant (p-value < 0.001) (Table-I).

Table-I: Comparison of Chronic Endometritis on H&E with CD138 (Immunohistochemistry)

CD138	H&E		Total	P-value
	Positive	Negative		
Positive	27	9	36	0.001
Negative	5	59	64	
Total	32	68	100	

Figure-I (A&B): A: H&E showing plasma cells in Endometrial Stroma (arrow highlighting plasma cells) B: Syndecan-1 showing numerous plasma cells in Endometrial Stroma



Discussion

Chronic Endometritis (CE) is a persistent/chronic inflammation of the endometrium and is observed in 3-10% of women who go through an endometrial biopsy for different medical conditions. Clinical presentation of Chronic Endometritis varies and most patients have mild symptoms such as abnormal uterine bleeding, pelvic pain, dyspareunia, and leucorrhea. Abnormal uterine bleeding is one of the

most common gynecological problems and has remained one of the persistent reasons for hysterectomy in developing countries. Abid et al. conducted a study on endometrial curetting specimens in 2014, in patients with complaints of AUB. The usual pathology identified was hormonal imbalance (27%), followed by endometrial polyp (14%), chronic endometritis (12%), atrophic endometrium (6%), endometrial hyperplasia (5%), and endometrial carcinoma (2%).[7] Various studies have disclosed that CE has a strong association with infertility.[8] It is prevalent in our country due to various reasons like lack of medical knowledge/facility, poverty, increasing antibiotic resistance, and/or due to different cultural beliefs. Approximately 18-20% of our population suffers from this. Shaheen et al. published a study in 2010, which was a cross-sectional study of Pakistani women and the prevalence of infertility was found to be around 7%.[8] A retrospective study from Pakistan of 3475 endometrial biopsies by Nisa et al. in infertile females showed chronic endometritis in 12% cases.[9]

CE is more prevalent in the reproductive age group. According to Cicinelli et al. mean age of CE in their population was 30.5±4.9 years.[3] Similarly it was 29±0.24 years in a recent study by Kozyreva et al.[10] In our study, the mean age group of patients diagnosed as CE was 33.4±4.3 years with minimum and maximum age of 20 and 44 years respectively. Histopathologically, the diagnosis of CE is based on the presence of plasma cells (PC) in the endometrial stroma. Other supportive factors include various morphological changes of stroma like spindling, edema, increased density, breakdown, chronic inflammatory infiltrate, and sometimes glandular asymmetry. Usually, PC cells have abundant basophilic cytoplasm, eccentric nucleus, "clock face" pattern of nuclear chromatin, and a visible perinuclear halo. However, their identification can be masked due to several reasons. Most importantly they are few, individually scattered, and have a maximum size of 5 μm. Certain stromal cells present in the surroundings also mimic PCs such as mononuclear cells.[11]

A study was done to evaluate the pathology of the endometrium after early reproductive losses (RL). A group of 306 women with early RL were prospectively examined for up to 6 months. The thickness, color, and structure of the mucosa of the uterus were studied hysteroscopically and then correlated with a number of plasma cells in succeeded biopsies both via H&E and CD138 stain method. The study concluded that immunohistochemical staining with CD138 showed a higher diagnostic accuracy of CE detection as compared to the classical morphological method.[12]

Kannar et al. performed a study in 2012, to determine the utility of syndecan-1 (CD138) in the diagnosis of CE in patients with abnormal uterine bleeding (AUB), and to see if any of the secondary histologic features in endometrial biopsy correlated with the presence of PC on IHC, and 50 samples were collected.

By using both light microscopy and immunohistochemistry, PC were searched and graded by selecting a hot spot area and calculated in ten high power fields. They were stratified as "negative" when there were no PCs, "1+" when less than 5 PCs were present, "2+" when 5-10 PCs were present, and "3+" when more than 10 PCs were present. They were also then correlated with different phases of endometrium like proliferative phase, secretory phase, endometrial polyps, and Disordered Proliferative Endometrium (DPE). Their study concluded that PC was seen in 11 (69%) of DPE, 8 (66%) of proliferative phase, and 2 (40%) of secretory endometrium. Syndecan-1 (CD138) was found helpful in those cases, where CE was suspected.[13]

In 2014, a retrospective cohort study was done to evaluate the distribution of inflammatory cell subpopulations within the endometrium of women with recurrent pregnancy loss and chronic endometritis (CE). Immunostains like CD138, CD56, CD163, CD14, CD20, and CD79a were performed. Expression of these markers was scored as: 0 = no PC, <1/hpf; 1 = 1-5 PC/HPF or clusters of less than 20 PC; 2 = 6-20 PC/HPF or clusters of at least 20 PC; 3 = >20 PC/HPF or sheets of plasma cells. The result showed that CE was found in 13% (14/107) of endometrial biopsies. The endometrium of subjects with CE demonstrated significantly greater numbers of PCs by CD138 staining compared to subjects without CE (p-value =0.013). There were no statistically significant differences in the number of B cells, NK cells, or macrophages in the

endometrium of women with and without CE. CD138 failed to highlight PCs in biopsies of patients who were treated with doxycycline for CE.[14]

Chen et al. conducted a study to investigate the role of CD138 immunohistochemistry in the diagnosis of CE and the risk factors which were associated with chronic endometritis in patients undergoing assisted conception, and 93 patients, with normal uterine shape confirmed by examination and who were planning to undergo assisted conception treatments, were selected as research subjects. Endometrial tissue was isolated for both H&E and CD138 immunohistochemical staining.

They used the criteria of diagnosing CE when 5 or more than PCs were present in a single HPF.[15] The result showed that the detection rate of CD138 immunohistochemical staining was greater than that of H&E staining (27.96% vs. 26.89%, *p*-value < 0.05). In our study, there were 32 patients who had positive endometritis on H&E and 36 patients had positive endometritis on CD138 staining. There were 27 true positives [diagnosed positive on both methods] and 59 true negative cases [diagnosed negative on both methods]. The *p*-value was significant (*p*-value < 0.001).

Conclusion

The current study confirms the increased sensitivity of CD138 immunohistochemical stain as a diagnostic tool for diagnosis of Chronic Endometritis by comparing it with Haematoxylin and Eosin stain.

Authors Contribution: UR: Design of work, Acquisition and analysis of data and Drafting. **NM:** Conception of work Interpretation of data and revising. **ZM:** Interptation of data and revising.

All authors critically revised and approve its final version.

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