EVALUATION OF ENDOMETRIAL BIOPSIES FOR CHRONIC ENDOMETRITIS BY USING SYNDECAN-1 (CD138) IN ABNORMAL UTERINE BLEEDING

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ABSTRACT

Objective: To determine the utility of Syndecan–1 (CD138) in the diagnosis of Chronic endometritis in patients with Abnormal Uterine Bleeding.

Study Design: Cross-sectional study**.**

Place and Duration: The study was carried out in department of Pathology, Army Medical College, Rawalpindi in collaboration with the department of Obstetrics & Gynecology, Pak Emirates Military Hospital (PEMH), Rawalpindi. The study duration was from Jan 2018 to Oct 2018.

Methodology: A total of one hundred endometrial biopsies of patients with clinical or histological suspicion of chronic endometritis were taken. Hematoxylin and Eosin (H & E) stain was performed on all the biopsies and a diagnosis was made. Later on, Syndecan-1 was applied on all the biopsies and an immunohistochemical diagnosis was made. Both the diagnoses were then compared and analyzed.

Results: Mean age of the study participants was 43.67 ± 8.95 years, with a range of 23-70 years. Out of 100 biopsies, forty-five cases (45%) were diagnosed as Chronic endometritis on H & E, while fifty-five cases (55%) were not showing Chronic endometritis and were diagnosed as disordered proliferative endometrium (DPE). On application of Syndecan-1 (CD 138), it was seen that these 19 out of 45 cases, diagnosed as Chronic endometritis on H & E were actually over-diagnosed and didn't show plasma cells. Out of 55 cases which were negative for Chronic endometritis, 15 cases were underdiagnosed as they showed plasma cells with immunohistochemistry application (Syndecan-1).

Conclusion: In cases suspected as Chronic endometritis, no plasma cells were clearly visible on H & E staining, Sydnecan-1 helps identifying plasma cells easily.

Keywords: Abnormal uterine bleeding, Chronic endometritis, Plasma cell, Syndecan-1.

INTRODUCTION

Abnormal uterine bleeding (AUB) is a common reason for women of all ages to consult their gynecologists. It includes both organic and non-organic causes of uterine bleeding[1]. AUB is defined as an excessive, erratic or irregular bleeding that does not correspond to the frequency, duration or amount of blood flow of a normal menstrual cycle[2,3]. It is considered one of the most challenging problem being faced by the gynecologists, regardless of the age of the women. The common pathologies associated with AUB in the adult females include Chronic Endometritis (CE) and Disordered Proliferative Endometrium (DPE). CE is the chronic and persistent inflammation of the endometrium[4]. It is observed in 3-10% of the women who present with AUB[5,6]. CE also usually occurs in 9-14% of women between menarche and menopause, which significantly has a great impact on quality of life and increasing the financial burden[3]. CE is usually asymptomatic or can be presented only with the subtle symptoms of abnormal uterine bleeding, pelvic pain, leucorrhea and dyspareunia. It can easily be ignored by the patients themselves and because of such a time-consuming microscopic examination, relatively mild manifestations and benign pathology. It is also prone to be missed in the clinical assessment both by the gynecologists and the pathologist[7]. CE is characterized histologically by the existence of plasma cells within the stroma of endometrium[4]. Other non-organic

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causes of CE in cases of AUB include pelvic inflammatory disease (PID), infections, presence of intrauterine device (IUD), retained products of conception (RPOC's) after delivery, elective abortion, uterine surgery and cervical stenosis[8-10]. Recent studies have also reported that CE is also associated with infertility and recurrent miscarriages[4]. Disordered proliferative pattern is also found in 20.5% of patients, irrespective of the age group and lies at one end of the spectrum of proliferative lesions of the endometrium that includes carcinoma at the other end with intervening stages of hyperplasia. It denotes an endometrial appearance that is hyperplastic but without an increase in endometrial volume and is a common and normal pattern in the peri-menarchal and perimenopausal years[1]. In its simplest form, scattered cysts were only moderately dilated, much as would be encountered in a disordered proliferative endometrium caused by unopposed estrogens of anovulationand exogenous estrogen therapy.

The most common methods of endometrial sampling in current clinical practice are outpatient pipelle endometrial biopsy, diagnostic dilatation and curettage (D & C) and hysteroscopy. Pipelle endometrial biopsy is now considered as first line diagnostic tool because of its accuracy, safety, convenience and efficacy. The secondary histological features that are associated with CE, like disparity between gland size, architectural irregularity of glands along with spindly and fibrotic stroma are also helpful[8]. On routine/conventional H & E staining, it is often difficult to distinguish plasma cells from fibroblasts, plasmacytoid stromal cells and mononuclear infiltrate within the endometrial stroma. Therefore, an immunohistochemical marker named Syndecan-1 (SDC 1), also designated CD 138, which is a transmembrane heparin sulfate proteoglycan, member of syndecan family and the only protein of diagnostic relevance is used to identify plasma cells. Under normal conditions, SDC 1 is expressed on cell membranes of mature plasma cells. Plasma cells have a characteristic appearance in which the chromatin is clock faced, with an eccentric nucleus and a visible perinuclear halo[13]. It is important to diagnose CE to eradicate the persistent infection, treat infertile patients resulting in successful pregnancy outcomes and prevent morbidity related to undue hysterectomies[5].

Normal tonsil tissue is recommended as control for SDC 1. It has a strong, predominantly membranous staining reaction. In addition, epithelial endometrial glands also show membranous positivity with SDC 1, which can be used as an inbuilt control[14]. Keeping in view the above background, the basic objective of this study was to assess the utility of Syndecan-1 in aiding the diagnosis of CE in patients with AUB.

METHODOLOGY

This cross-sectional study was carried out in the department of Pathology, Army Medical College, National University of Medical Sciences (NUMS) Rawalpindi in collaboration with the department of Obstetrics & Gynecology, Pak Emirates Military Hospital, Rawalpindi. The duration of study was from January to October 2018. A total of 100 samples of endometrial pipelle biopsies were enrolled in the study by non-probability convenience sampling. The sample size (n): 10013 was calculated by WHO sample size calculator and by taking Confidence level=95%, Anticipated population proportion *p (*7%) and Absolute precision required D (5%). The endometrial biopsies having unequivocal evidence of CE with clearly appreciable plasma cells on H & E staining, or showing some other obvious pathology like granulomatous endometritis, endometrial hyperplasia or carcinoma were excluded from the study. The endometrial biopsies with clinical or histological suspicion of CE, with no plasma cells clearly appreciable on routine H & E staining and no other obvious pathology noted on histological examination, were included in the study.

On the basis of inclusion/exclusion criteria, the cases were diagnosed as chronic endometritis and no chronic endometritis/disordered proliferative endometrium on H & E stain. Later on, Syndecan-1 was applied and an immunohisto-

chemical diagnosis was made. The cases which were diagnosed as chronic endometritis both on H & E and after immunohistochemistry (IHC) were labelled as true positive. Those which were diagnosed on H & E as chronic endometritis and proved disordered proliferative endometrium after IHC were labelled false positive, those which were diagnosed both on H & E and IHC as disordered proliferative endometrium as true negative and those diagnosed on H & E as disordered proliferative endometrium and on IHC as chronic endometritis as false negative. Data analysis was computer based using SPSS version[23]. Frequencies and percentages were calculated for qualitative data and mean and standard deviation were calculated for quantitative data. Chi square Fisher exact test was applied to compare both the opinions that were made on H & E and after immunoreactivity with Syndecan-1. A *p*-value of ≤0.05 was considered statistically significant.

Table-I: Histological features of Chronic Endometritis on H & E.

Histological features	Opinion on H&E	
	CE	DPE
Disparity between gland size	45	55
Architectural irregularity of glands	45	55
Spindly & fibrotic stroma	45	-

Table-II: Comparison of opinions made on H & E and IHC (Syndecan-1).

	Opinion on H&E (n=100)	Opinion on IHC (SDC1) (n=100)	*p*-value
Chronic endometritis	45	41	0.002*
Disordered Proliferative Endometrium	55	59	

*p value ≤0.05 statistically significant

RESULTS

A total of one hundred endometrial pipelle biopsies 100 of patients with AUB were included in the study which were considered adequate fulfilling the set parameters established in inclusion and exclusion criteria. Mean age of the study participants was 43.67 ± 8.95 years, with range from 23 to 70 years. Firstly, all the slides which were stained with H & E were examined on low and high power by using light microscope. Out of 100 biopsies, forty-five cases (45%) were diagnosed on H & E as chronic endometritis (table-I & II). The additional features like disparity between gland size and architectural irregularity of glands were seen in all the cases of CE (45%) and DPE (55%) on H & E. Whereas, spindly and fibrotic stroma was seen exclusively in CE (45%) biopsies on H & E examination (table-I). Fifty-five cases (55%) were diagnosed as no chronic endometritis/disordered proliferative endometrium. Later on, immunohistochemistry

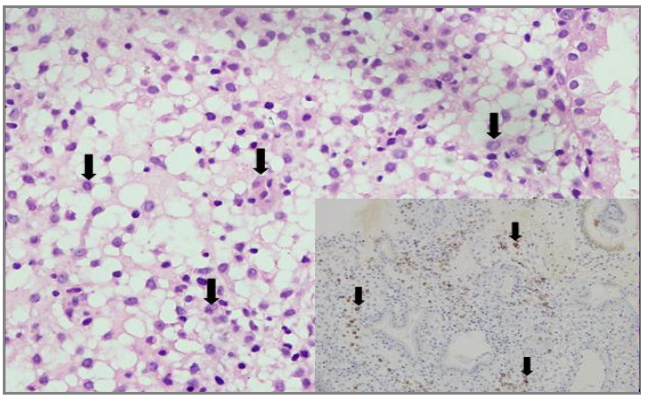

Figure-1: Photomicrograph showing few plasma cells (Black arrows) in spindly stroma of endometrial biopsy on H & E stain. (High Power View). Inset showing plasma cells (Black arrows) after immunostaining with Syndecan-1 (True Positive).

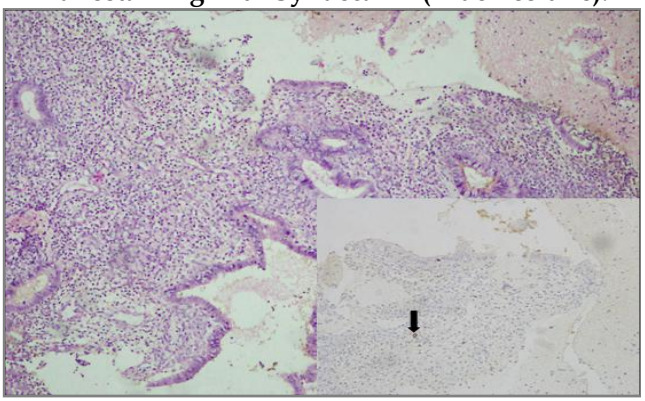

Figure-2: Photomicrograph of endometrial biopsy showing irregular architecture and size, diagnosed as Disordered Proliferative Endometrium on H & E. Inset showing plasma cell (Black Arrow) after immunostaining with Syndecan-1, so it was actually CE (False Negative).

(Syndecan-1) was applied on all cases. The normal tonsil tissue was run as control with all the slides. Out of 45 biopsies which were initially given the diagnosis of chronic endometritis on H & E, 26 biopsies were true positive (fig-1) and 19 biopsies were false positive. Out of other 55 cases, which were diagnosed as, no chronic endometritis/disordered proliferative endometrium on H & E, showed 15% false negative cases (fig-2) and 40% cases as true negative. All cases diagnosed as chronic endometritis showed very clear syndecan-1 immunoreactivity on the cell membranes of plasma cells. A *p*-value=0.002 was obtained, which was considered statistically significant.

DISCUSSION

Pipelle endometrial biopsy is an important diagnostic tool in the investigation of abnormal uterine bleeding which had replaced the conventional dilatation and curettage (D & C). This technique is now considered as first line diagnostic tool because of its accuracy, safety, convenience and time saving[11,12]. In a study performed at Ohio State University, 26 of 157 (16.6%) female patients included in the study had chronic endometritis[13,14]. In another study performed at University of Edinburgh, United Kingdom, chronic endometritis was detected in 52.94% of the endometriosis group and 27.02% of the non-endometriosis group[4]. It has been reported by Feghali and coworkers that chronic endometritis is associated with infertility and recurrent abortion and it has been identified in 45% of the infertile patients[15,16]. In a study carried out in Institute of Pathology, Faculty of Medicine, University of Bari and the Department of Obstetrics and Gynecology, Hospital of Rimini, Italy, chronic endometritis at histology was found in approximately 30% of infertile women and 35% of women presenting with abnormal uterine bleeding[17]. In a study carried out in 2010, at the Center for Advanced Reproductive Services, Department of Obstetrics and Gynecology, University of Connecticut Health Center, Farmington, Connecticut by Johnston–MacAnanny and co-workers found that chronic endometritis was identified in 30.3% of patients with recurrent implantation failure (RIF)[18]. Another study done at 3rd Unit of Obstetrics and Gynecology, Department of Biomedical and Human Oncological Science (DIMO), University of Bari, Italy showed 70 out of 106 (66.0%) women were diagnosed with chronic endometritis at hysteroscopy. In 61 (57.5%) chronic endometritis was confirmed by histology and 48 (45.0%) by cultures. Common bacteria and mycoplasma were the most prevalent agents[19]. The prevalence of CE in women with RIF in this study (66.0%) was about double compared with 30.3% reported by Johnston-MacAnanny[18]. In present study, the prevalence of chronic endometritis in abnormal uterine bleeding was 26% which were true positive cases and 15% which were false negative cases, making it 41% of all the cases, which were comparable to that reported by Cicinelli[19]. Although the prevalance of CE can be biased by the characteristics of the patients who visited the clinic, present study was limited in this way as we didn't include the etiologies behind CE and it was actually confined to immunohistochemistry. Chen and co-workers performed a study in 2016, in the department of Gynecology & Obstetrics and department of Pathology, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong Province, People's Republic of China, in which positive rate for CE of the H & E group was 0 and the suspected positive rate was 26.89% 25/93. Both rates were lower than the positive rate of the CD 138 immunohistochemistry group (27.96%, 26/93), and the difference was statistically significant (p=0.041)[20]. Another study performed at University of Arkansas for Medical Sciences, Little Rock, Arkansas by ilene and coworkers found that all the cases of CE showed clear immunoreactivity on plasma cell membranes[5]. In our study 26% of cases were true positives which were suspected as CE and they turned out to be CE. In addition, 15% of cases turned out to be CE after staining with Syndecan-1, which were initially diagnosed as disordered proliferative endometrium. Thus, in total 41% of biopsies were confirmed after immunohistochemistry which were comparable to that mentioned by Chen and co–workers[20]. In

summary, the amount of time spent looking for plasma cells in cases suspicious for chronic endometritis staining was reduced with syndecan-1. It was helpful in diagnosing those cases in which other histological findings hinder the search for plasma cells. The importance of using Syndecan-1 is proved to avoid wrong diagnosis since the plasmacytoid stromal cells and other mononuclear inflammatory cells do not stain with syndecan-1. It specifically stains only plasma cells, thus preventing any possibility for false positive results in diagnosing chronic endometritis.

RECOMMENDATION

Thus, it is recommended to use Syndecan-1 for diagnosis of chronic endometritis, especially in cases where no plasma cells are easily identifiable on H & E stained slides, however, there is a strong clinical or histological suspicion of chronic endometritis.

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CONCLUSION

In conclusion, the doubtful cases in which search of plasma cells was limited on examination of H & E stained slides and hampered by histological differentials like disordered proliferative endometrium, immunohistochemistry for Syndecan-1 can be an efficacious ancillary test in recognition of plasma cells for definite diagnosis of chronic endometritis.

CONFLICT OF INTEREST

There was no conflict of interest to be declared by any authors.

REFERENCES

1. Doraiswami S, Johnson T, Rao S, Rajkumar A, Vijayaraghavan J, Panicker VK. Study of endometrial pathology in abnormal uterine bleeding. J Obstet Gynecol India 2011; 61(4): 426-30.
2. Al-Neaimy WMT, Ahmed MT, Al-Jawadi SI. Histopathological Interpretation of Abnormal uterine bleeding after the age of 40 year. Iraqi Postgrad Med J 2010; 9(3): 274-82.
3. Rifat AG, Mahmoud MM. Endometrial Histopathological changes in women with Abnormal Uterine bleeding in Kirkuk City, a Clinicopathological Study. Med JBabylon 2013; 10(3): 567-82.
4. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. The association between endometriosis and chronic endometritis. PLoS One 2014; 9(2): e88354-60.
5. Bayer-Garner IB, Korourian S. Plasma cells in chronic endometritis are easily identified when stained with syndecan-1. Mod Pathol 2001; 14(9): 877-79.
6. Naji AZ. Enhance and prove diagnosis of chronic endometritis with CD-138 immunostain. Med JBabylon 2012; 9(3): 598-03.
7. Kitaya K. Prevalence of chronic endometritis in recurrent miscarriages. FertilSteril 2011; 95(3): 1156-58.
8. Gilmore H, Fleischhacker D, Hecht JL. Diagnosis of chronic endometritis in biopsies with stromal breakdown. Hum Pathol 2007; 38(4): 581-84.
9. Achilles SL, Amortegui AJ, Wiesenfeld HC. Endometrial plasma cells: do they indicate subclinical pelvic inflammatory disease? Sexually transmitted diseases 2005; 32(3): 185-88.
10. Eckert LO, Hawes SE, Wölner-Hanssen PK, Kiviat NB, Wasserheit JN, Paavonen JA, et al. Endometritis: the clinical-pathologic syndrome. Am J Obstet Gynecol 2002; 186(4): 690-95.
11. Mutter GL, Bergeron C, Deligdisch L, Ferenczy A, Glant M, Merino M, et al. The spectrum of endometrial pathology induced by progesterone receptor modulators. Mod Pathol 2008; 21(5): 591-98.
12. Samson S-L, Gilmour D. Who needs an endometrial biopsy? Can Fam Physician 2002;48:885-87.
13. Kannar V, Lingaiah HKM, Sunita V. Evaluation of endometrium for chronic endometritis by using syndecan-1 in abnormal uterine bleeding. J Lab Physicians 2012; 4(2): 69-73.
14. O'connell FP, Pinkus JL, Pinkus GS. CD138 (syndecan-1), a plasma cell marker: immunohistochemical profile in hematopoietic and nonhematopoietic neoplasms. Am J Clin Pathol 2004; 121(2): 254-63.
15. Knudtson EJ, Shellhaas C, Stephens JA, Senokozlieff M, Ye H, Iams JD. The association of chronic endometritis with preterm birth. Am J Obstet Gynecol 2007; 196(4): 337.e1-4.
16. Feghali J, Bakar J, Mayenga J, Segard L, Hamou J, Driguez P, et al. Systematic hysteroscopy prior to in vitro fertilization. Gynecologie, Obstetrique&Fertilite 2003; 31(2): 127-31.
17. Cicinelli E, Resta L, Nicoletti R, Tartagni M, Marinaccio M, Bulletti C, et al. Detection of chronic endometritis at fluid hysteroscopy. J MinimInvasive Gynecol 2005; 12(6): 514-18.
18. Johnston-MacAnanny EB, Hartnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil Steril 2010; 93(2): 437-41.
19. Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Human Reproduction 2014; 30(2): 323-30.
20. Chen YQ, Fang RL, Luo YN, Luo CQ. Analysis of the diagnostic value of CD138 for chronic endometritis, the risk factors for the pathogenesis of chronic endometritis and the effect of chronic endometritis on pregnancy: a cohort study. BMC women's health 2016; 16(1): 60-66.