

Research Article

Chronic endometritis in *In-Vitro* fertilization failure patients

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Abstract

Introduction: Chronic Endometritis (CE) is a common cause of infertility in asymptomatic patients and its diagnosis and treatments improved assisted reproduction technique outcome in most of the specialized centers. Diagnosis of CE in endometrial biopsy by Hematoxylin and Eosin (H&E) stain is hard to identify chronic inflammatory cells from the stroma and the use of plasma cells-specific stains is helpful.

Aim of the work: Evaluation of the use of CD138 in the identification of plasma cells in endometrial biopsy of patients with previous IVF trial failure.

Material and methods: Hysteroscopic and curettage endometrial biopsies from fifty-five females with previous IVF trial failure were stained with H&E and CD138 immunostaining for detection of plasma cells.

Results: Plasma cells were identified in 52.7% of cases by H&E and in 6/55 by CD138 immunostaining. CD138 is more sensitive in detecting plasma cells in endometrial biopsy than H&E stain. There was a significant statistical correlation between CE and abnormal uterine bleeding, abortion and primary infertility ($P > 0.5$).

Conclusions: Diagnosis of CE is helpful in infertility patients with IVF trial failure to improve the outcome of the maneuver. CD138 is more sensitive for plasma cells especially in endometrial biopsies than H&E.

Introduction

In spite of the fact that disordered uterine function is considered to have a major impact on the possibilities to conceive through IVF, conventional infertility investigations, including ultrasound and Hysterosalpingography (HSG) may miss subtle intrauterine lesions. After hysteroscopy maneuver before performing IVF, the incidence of undiagnosed intrauterine lesions has been demonstrated to be at the range of 11 - 45% [1]. One of these abnormalities is Chronic Endometritis (CE) that is often asymptomatic or can be accompanied by non-specific symptoms. The use of histopathology in detection of plasma cells in the endometrial curettage is essential for the diagnosis of CE [2].

Chronic endometritis may interfere with receptivity of the endometrium and therefore, leads to infertility because CE is characterized by an abnormal pattern of lymphocytic infiltration and consequently, an abnormal endometrial microenvironment [3]. In a study conducted in 2014, it was demonstrated that CE is a frequently found in females with repeated abortions,

and consequently, women who took antibiotic therapy had a significantly higher rate of successful pregnancies compared to those without treatment [4]. Mainwile, CE was a pathological diagnosis in about 30% of females with repeated implantation failure at IVF and those that were diagnosed as CE had about (11.5%) lower rates of implantation after an IVF cycle [5].

On the other hand, Kasius, et al. reported a minimal clinical implication of CE on fertility after IVF and they documented CE in only (2%) of asymptomatic women with infertility. They concluded that pregnancy rate after IVF was not affected by CE [6].

Syndecan-1 or CD138 is a well-known trans-membrane heparan sulfate proteoglycan family member, which acts as a receptor at extracellular matrix and is involved in different cellular functions, mainly cell-cell and cell-matrix adhesion. Expression of CD138 is observed normally on the surface of mature epithelial cells; however, stromal expression was also detected in developing tissues. In the hematopoietic system, expression of CD138 is thought to be restricted to normal and



malignant plasma cells. Based on messenger RNA studies, high levels of CD138 was restricted to the precursor B-cell and plasma cell stages of B-cell differentiation [7].

B-cells constituted about <1% of all leukocytes in normal endometrium. B-cell infiltration is rarely detected, in the functional layer of the endometrium but is found mainly in the basal layer, as central cells in the lymphocyte aggregates are surrounded by macrophages and many CD8+ T-cells. In chronic non-specific endometritis, a large number of B-cells focally infiltrates both the endometrial stroma and glandular epithelium [8].

Accumulating evidence documented the efficiency of antibiotic oral therapy to eliminate endometrial stromal plasma cells in patients with CE. On the other hand, it is unclear to the investigators on how antibiotic treatment changes endometrial microbial profiling in CE. Several studies documented the abnormal expression of many pro-inflammatory chemical mediators involved in B-cell extravasation in CE. Selectin plays an important role in tethering/rolling of circulating B-cells on endothelial cells. While Selectin E is not detected in normal endometrium, in CE endometrial vascular endothelial cells express this B-cell adhesion molecule. Moreover, endometrial microvascular endothelial cells specifically expressed CXCL-13 in CE, which is a chemo-attractant that activates the adhesion molecules on B-cells and endothelial cells. Endometrial glandular epithelial cells with CE also expressed CXCL-1, a chemokine involved in B-cell migration [9]. All these findings suggest that local microbial infection in the endometrium stimulates the immune responses, which potentially retrieve circulating B-cells into the endometrial stromal and the glandular areas. The migrating B-cells may differentiate into plasma cells within the endometrial stroma. It was found that endometrial stromal plasma cells express multiple immunoglobulin subclasses (IgM, IgA1, IgA2, IgG1, and IgG2) with a predominant IgG2. These excessive mucosal antibodies affect the process of embryo implantation negatively [10].

Endometrial micro polyposis was reported to be associated with endometrial stromal edema, thickening, and periglandular hyperemia noticed with CE. These lesions cannot be detected by ultrasonography or hysterosalpingography but can be diagnosed by fluid hysteroscopy. Hysteroscopy therefore has an important role in the diagnosis of CE in Gynecology and Obstetrics practice. On the contrary, local bacterial examination alone is unlikely to be useful in the diagnosis of CE [11].

A small number of plasma cells in the endometrial curettage biopsy are difficult to be identified in the H&E-stained sections. In addition, the histological changes in the proliferative and secretory phases of endometrium can also interfere with the detection of plasma cells and the diagnosis of CE. During late menstruation or early proliferative endometrium, significant lymphocyte infiltrations, mitosis in the stroma, and plasma cell-like stromal cells, appear in the endometrial tissue. These can simulate plasma cells in the endometrial tissue and interfere with the diagnosis of CE [12].

Aim of the work

To study the use of immunohistochemical expression of CD138 in the diagnosis of Chronic Endometritis (CE) which is considered as a risk factor for assisted reproduction (IVF) failure patients.

Materials and methods

In order to uncover the controversial role of CE in IVF failure women, we evaluated the incidence of CE by both hysteroscopy and histopathology in 55 women who experienced IVF-failure (failure to get pregnant). The study protocol was accepted by the Sohag Medical ethical committee and every female signed a written consent for the maneuvers (both hysteroscopy and endometrial biopsy that were performed as a part of workup for IVF-ET second trial). The cases, who have been diagnosed with CE in the study were given the appropriate antimicrobial drugs and were given a period of 6 months to 1 year before starting another trial of IVF. Specific exclusion criteria of the study cases include: 1. Females with intrauterine contraceptive devices, (as it is characterized by prolonged plasma cell accumulation even after their removal from the uterine cavity). 2. Endometriosis. 3. Post-gestational long-term retention of products of conception. 4. Acute suppurative endometritis (recognized as neutrophil invasion and micro abscess formation in the endometrium). 5. Uterine and/or cervical tumors. 6. Previous recent treatments with chemotherapy. 7. Specific disease as hypothyroidism, tuberculosis, diabetes, liver or renal chronic diseases. After hysteroscopy, endometrial biopsies were collected from 55 cases of IVF trial failure females, specifically from the endometrial micro polyposis areas. It was fixed immediately by immersion in 10 % neutral formaldehyde for fixation. After ethanol gradient dehydration, the endometrial tissues were cleared with Xylene, immersed in paraffin for embedding, and 4 µm serial sections were cut. All paraffin blocks were stained with routine H&E and Geimsa stains for detection of plasma cells and diagnosing CE (Geimsa stain was used to confirm the diagnosis of CE and the presence of plasma cell detected by H&E as the plasma cells were stained red in color with Geimsa stain for easy detection). Paraffin-embedded, endometrial tissue blocks were cut and mounted on 3-aminopropyl-triethoxysilane-coated slides and stained using Syndecan-1 (monoclonal mouse antihuman CD 138; Clone M115, Dako) at a dilution 1/50 for 2 H, at room temperature). The immunostained slides were scored for the presence of membranous immunostaining of plasma cells using light microscopy, in at least 10 HPF. Each section was graded as Negative (-): when no plasma cells stained with CD138; Positive (+) when <5 plasma cells were detected; (++) when 5-10 plasma cells were detected, and (+++) when >10 plasma cells were stained (13). The secondary histologic features of CE like gland architectural irregularity, spindling of the stroma, stromal edema and/or hemorrhage with the presence of plasma cells were considered. All immunohistochemical and H&E findings were statistically analyzed using SPSS software version 14 and Fischer's exact tests. Value of P < 0.05 was considered significant [14].

Results

Fifty-five women with a history of previous IVF failure were included in this study with age range 18–45 years with mean age 31.5 years. Twenty-seven cases were diagnosed as primary infertility, while 28 cases complained of secondary infertility. Proliferative endometrium was seen in 32 cases and secretory endometrium was seen in 23 cases. Seven cases had previous abortions, and 27 cases complained of prolonged abnormal uterine bleeding. CE was diagnosed in 29/55, (52.7%) by H&E and Geimsa stains and in 6 cases (10.9%) by CD138 immunostaining. A previous history of abnormal uterine bleeding episodes, abortion and primary infertility due to fallopian tube obstruction were statistically correlated with CE ($P < 0.05$). CD138 immunostaining expression had no statistically significant correlation with age of the patient ($p < 0.7$), secondary infertility ($P < 0.05$), or the type of endometrium either proliferative or secretory ($P < 0.6$). CD138 is a more sensitive test (sensitivity 66.7%) but less specific for plasma cells (specificity 48.9%) while routine H&E staining was detected to be less sensitive than CD138 (sensitivity 55.7%) and equal to CD138 as specific for plasma cells detection (specificity 50%). The use of H&E together with Geimsa stain is sensitive and specific for detection of plasma cells in endometrial biopsy as CD138 Tables 1,2 and Figures 1,2.

Discussion

Infertility is a relatively common gynecological problem. Relevant studies have shown that the incidence of chronic endometritis (CE) in infertile patients ranges from 0.2–46 % [15].

Chronic endometritis (CE) is a local non-specific inflammatory disease characterized by abnormal plasma cells infiltration in the endometrial stromal areas. In the present

Table 1: The clinicopathological characteristics of the study groups.

	NO	CE + with H&E	CE+ with CD138	P value
Age range	20-45			
1ry Infertility	27	13	3	0.03
2dry infertility	28	16	3	0.06
Tubal factor	19	13		0.005
Previous miscarriage	7	4		0.005
AUB	27	16		0.002
Proliferative E	32	16	3	
Secretory E	23	13	3	
CE		29(52.7%)	6/55(12%)	

Table 2: The sensitivity and specificity of both H&E and CD128 in detection of plasma cells in CE.

	H&E	CD138
Sensitivity	55.7%	66.7%
Specificity	50%	48.9%
+ve predictive value	52.7%	13.8%
-ve predictive value	89.7%	92.3%

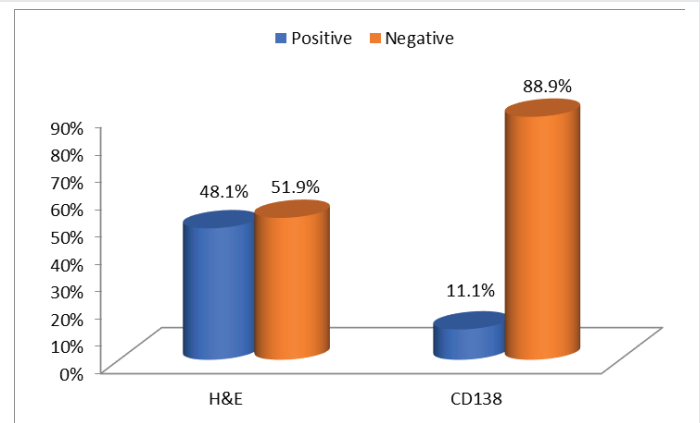


Figure 1: The representation of H&E and CD138 positive plasma cells in 1ry infertility cases.

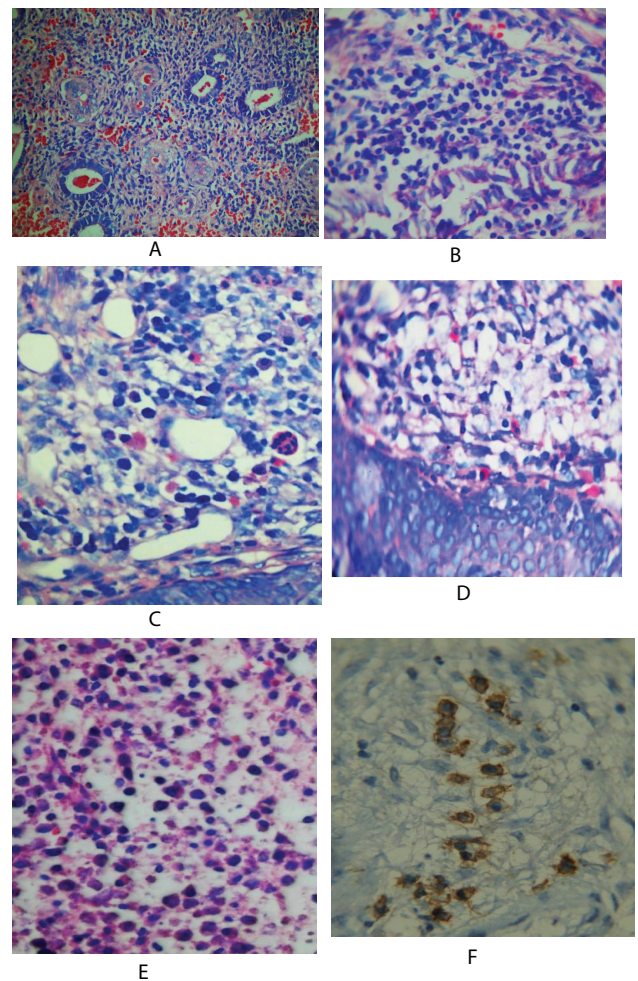


Figure 2: A proliferative endometrium with CE (H&E) X 40). B: Endometrial infiltrate with B-cells stained by Geimsa stain (X 100). C: D: CE with plasma cells stained red by Geimsa stain (X 100). E: Plasma cells stained with H&E in a case with previous abortion (X 200). F: Plasma cells membranous immunostaining by CD138 (X 200).

study CE was detected by H&E in 52.7% (29/55) and in 12% by CD138 of the studied cases that experienced implantation failure after IVF trial. There was a significant statistical correlation between CE and specific risk factors as abnormal uterine bleeding, previous abortion, and primary infertility caused by tubal factor ($p > 0.5$).



Recent studies have disclosed the association between CE and reproductive failure. Chronic endometritis was identified in 30% of the patients with repeated implantation failure after *in vitro* fertilization-embryo transfer, in 28% of the patients with unexplained infertility, and in 12% of the patients with unexplained recurrent miscarriages [16-18].

An earlier study on the underlying causative agents in CE found that the microorganisms detected more frequently in the endometrium with CE are common bacteria [Streptococcus species (27%), *Escherichia coli* (11%), *Enterococcus faecalis* (14%)], mycoplasma species (*Mycoplasma Genitalium* (15%) and *Ureaplasma Urealyticum* (11%). The bacterial flora in the endometrial tissue culture was inconsistent with those in the vaginal or endocervical swab culture in patients with CE [19].

In the present study a mixture of bacterial (Streptococci species), Fungal (*Candida Albicans*) and viral (HPV) were detected in the cervical swab examination of the studied cases but none of these showed a statistically significant correlation with CE. A recent study demonstrated that CE is a common finding in women complaining of repeated unexplained implantation failure. The reproductive outcome at IVF was significantly improved in those patients in whom antibiotic treatment was able to normalize both hysteroscopic and histologic endometrial pattern [20].

In agreement with our results, it was suggested in a recent study in 2016 that a previous prolonged menstrual bleeding episode, an abortion history or fallopian tube obstruction were associated with the pathogenesis of chronic endometritis and were independent risk factors of CE. They have shown that among infertile patients, the prevalence of CE was 27.96 % and among patients who received assisted reproductive technology, the pregnancy rate in the control group (31.3 %) was significantly higher than that of the chronic endometritis group (7.7 %) ($P=0.017<0.05$), indicating that chronic endometritis may be one of the causes of infertility [12]. Chronic endometritis was detected in 14% of women with repeated implantation failure and in 27% in women with recurrent pregnancy losses in a more recent study in 2016 [21].

Although the association between CE (plasma cells infiltration of the endometrium) and abnormal uterine bleeding still remains to be established, there was a statistically significant correlation between CE and abnormal uterine bleeding ($P>0.5$) in our study. It was demonstrated that endometrium with CE uniquely expresses the chemokines CXCL-1, CXCL-13 and adhesion molecules Selectin E, implicating that local B-cells are recruited from endometrial microcirculation and differentiate *in-situ* into plasma cells. Such unusual leucocyte composition in CE may disrupt the integrity of the epithelial lining and cause endometrial shedding resulting in Abnormal Uterine Bleeding (AUB) [17].

In the present study, CE was detected in 52.7% of cases which is considered to be high rate compared to a cohort study of 395 cases of infertility with a history of two or more early pregnancy losses, CE was found in 9% of cases and the cure rate was 100% after a course of antibiotics and subsequent

live birth rate was 88% [22]. Another study found that CE was detected in 30.3% of patients with recurrent implantation failure and they concluded that recurrent implantation failure warrants investigations on CE as a contributing factor [16]. This discrepancy of CE incidence rate can be explained on the bases of a lower socioeconomic standard in most of the developing countries including Egypt that did not provide regular medical surveillance programs for early diagnosis and treatment of CE especially in asymptomatic infertile women.

In the present study CD138 showed sensitivity and specificity for detection of plasma cells in endometrial biopsy of 66.7% and 48.9% respectively in agreement with a similar study that evaluated Syndecan-1 (CD138) immunohistochemical staining of endometrial biopsies in 107 women with repeated pregnancy losses. They concluded that CD138 provided increased sensitivity for chronic endometritis diagnosis compared to H&E staining [22].

On the other hand, a prevalence of CE in 606 patients with an adequate biopsy was 2.8% in an earlier study with a cumulative live birth rate (including spontaneous pregnancies) did not significantly differ between patients with or without CE (76% versus 54%) [2].

Diagnosis and treatment of CE significantly improves implantation rate in patients undergoing IVF-ET (*In Vitro* Fertilization and Embryo Transfer) in a study that concluded CE to be found in 40.7% (11/27) of women [23] While in an earlier study, CD138-positive plasma cells were detected in 18 cases of CE while none of the 25 cases of abnormal uterine bleeding showed immunostaining for CD138 [24]. Kannar, et al. diagnosed CE in 3/50 cases by H&E and in 22/50 cases by immunohistochemistry technique [25], while in Hartman, et al. case plasma cells were stained by H&E in 29/100 and by CD138 in 90/100 cases of CE [26]. The different and variable correlation between CE and infertility risk factors as abnormal uterine bleeding in the studies necessitated a further work with larger groups of patients using cohort studies.

Conclusion

- Syndecan-1 (CD138) is a sensitive immunohistochemical marker for detection of plasma cells in CE than the routine H&E and Geimsa stains especially in asymptomatic infertility patients. Larger cohort studies are needed in the future to evaluate the role of CE in patients with IVF and implantation failure to improve the outcome of these techniques.

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