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DIAGNOSTIC EVALUATION OF UNEXPLAINED INFERTILE WOMEN FOR CHRONIC ENDOMETRITIS BY ENDOMETRIAL HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

Obstetrics & Gynaecology

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ABSTRACT

Introduction: Wide variety of unexplained infertility is due to Chronic Endometritis(10-66%). Diagnosis is based on detection of plasma cell infiltration in the endometrial stroma on histopathology. IHC staining for CD 138 improves the sensitivity. **Objective:** Percentage of chronic endometritis among Indian unexplained infertility by endometrial histopathology and immunohistochemistry. **Methods:** A total of 75 study subjects were enrolled in the study. Endometrial biopsy was taken in late follicular phase. The biopsy tissue was sent for histopathological examination for detection of plasma cells, for common bacterial cultures and for GeneXpert for detection of endometrial tuberculosis. The patients diagnosed with chronic endometritis were given antibiotic treatment according to the microbial cultures. **Results:** 12.2% cases (9/75) of unexplained infertility were diagnosed as chronic endometritis out of which 2 cases were diagnosed by routine histopathology and additional 7 cases were diagnosed by immunohistochemistry CD138. Detection by IHC CD138 was found to be much higher than routine HPE and the difference was statistically significant (12.2% versus 2.7%, p value = 0.008). Endometrial culture was positive in all CE patients showing growth of common organisms who received antibiotics according to the culture. One patient was diagnosed with TB endometritis. **Conclusions:** We detected chronic endometritis on endometrial biopsy in 12.2% of the unexplained infertility cases in Indian population. Immunohistochemistry for CD138 was found to be much superior to routine histopathology in diagnosing chronic endometritis. Tubercular endometritis was detected in only one case (1.3%) in our study population.

KEYWORDS

Unexplained infertility, Chronic endometritis, Immunohistochemistry CD 138, Intrauterine insemination

INTRODUCTION

Unexplained infertility may be diagnosed in 8% to 30% of infertility[1]. Studies have shown that a wide variety of unexplained infertility is due to chronic endometritis. Chronic endometritis impairs fertility by altering endometrial receptivity[2,3]. Higher conception rates are reported after treatment of chronic endometritis in infertile women. Diagnosis is based on detection of plasma cell infiltration in the endometrial stroma on histopathology. Immunohistochemistry staining for CD 138 (a proteoglycan found on cell surface of Plasma cells) improves the sensitivity for diagnosing CE[4,5]. There is a wide variation in reported prevalence of CE in endometrial specimens (10-66%). Not much study has been done on prevalence of CE in Indian population. This study aimed to find out the percentage of chronic endometritis among Indian unexplained infertility. Tubercular endometritis is also an important cause of infertility in developing countries like India. Various studies have reported prevalence of Female Genital TB to be between 15-20% among infertile women[6,7].

Aims and Objective

The present study aimed to detect chronic endometritis in unexplained infertility by histopathology and immunohistochemistry for CD138. The primary objective was to determine the percentage of chronic endometritis in unexplained infertility diagnosed by endometrial histopathology and immunohistochemistry for CD138. The secondary objectives were to determine the percentage of chronic endometritis on microbial cultures of endometrial biopsy sample, to determine the percentage of tubercular endometritis in unexplained infertility and to correlate the findings of routine histopathology, immunohistochemistry and microbial cultures.

MATERIALS AND METHODS

It was a single center Observational cross sectional study conducted from January 2021 to June 2022. A total of 75 study subjects were enrolled. A detailed history, examination and investigations were recorded on predesigned case record form. Written informed consent was taken from all the participants. Sample size was calculated considering prevalence of chronic endometritis in unexplained infertility as 56.8% in study conducted by Ettore Cicinelli et al,[1] and estimating relative difference of 20% on either side.

Inclusion Criteria were married women with unexplained infertility attending Gynae OPD/infertility clinic were included in the study. Age 18-40 years, normal husband semen analysis, normal tubal patency, regular menstrual cycle on history and ovulation documented on USG and normal ovarian reserve by normal Antral Follicle Count (AFC). Exclusion Criteria were uterine abnormalities like fibroids, polyp >5mm, uterine malformations, signs and symptoms of acute Pelvic inflammatory diseases (PID), presence of hydrosalpinx, vaginal infection, autoimmune conditions, active Tuberculosis and severe medical conditions where pregnancy is contraindicated.

Patients were called on day 9- 14 (late Follicular Phase) for endometrial biopsy. Endometrial biopsy was taken by Endometrial Curette. The tissue collected divided into three parts: One sample for histopathology and immunohistochemistry CD138 for detection of plasma cells, one sample for common bacterial cultures and third sample for GeneXpert for detection of endometrial tuberculosis and sent to the respective department. Cases diagnosed as chronic endometritis were given antibiotic treatment as per antibiotic sensitivity. Specific antibiotic were given according to antimicrobial sensitivity. All the cases of unexplained infertility were offered OI with IUI as per departmental protocol.

Statistical Analysis

Data were recorded in MS Excel spreadsheet program. SPSS statistical software version 23.0 was used. Descriptive statistics were expressed as mean +/- standard deviations and medians for continuous variables, and frequencies and percentages for categorical variables. Chi-Square test and McNemar test were used to evaluate concordance between immunohistochemistry, routine histopathology and microbiological culture. P-value<0.05 has been considered as significant.

OBSERVATIONS AND RESULTS

The demographic characteristics of the study population (unexplained infertility cases) were studied. Majority of the patients were in the age group 26-30 years, having normal BMI and belonging to lower middle class.

The mean duration of infertility was 3.05 years. 42 patients (56.0%) had Primary infertility and 33 patients (44.0%) had Secondary Infertility. All 75 patients underwent Endometrial Biopsy in the

Follicular Phase (day 11-13 of menstrual cycle) without anaesthesia.

Table 1: Histopathology Diagnosis Of Endometrial Biopsy (H & E Staining)

Histopathology	Frequency (n=74)	Percentage
Normal Proliferative Endometrium	72	96.0%
Chronic Endometritis	2	2.7%
Granulomatous Endometritis	1	1.3%

On routine HPE by H&E staining, 2 patients (2.7%) were diagnosed with CE and 1 patient was having Granulomatous Endometritis. (Table 1)

Table 2: Histopathology Immunohistopathology For CD138 Result

IHC CD138	Frequency (n = 74)	Percentage
Positive	9	12.2%
Negative	65	87.8%

By IHC CD138 staining on histopathology, in 9 (12.2%) cases, plasma cells were detected in EB samples and thus were diagnosed with Chronic Endometritis. (Table 2) The 2 cases diagnosed as CE on routine HPE were also diagnosed on IHC staining. Also 7 more cases were diagnosed as CE on IHC CD138 staining which were not detected by routine HPE. Thus on histopathology (routine & IHC) 12.2% cases of unexplained infertility were diagnosed as chronic endometritis.

Table 3: Correlation Between Routine HPE and IHC CD138

	Category			Chi-Square Test	
	Routine HPE	IHC CD138	Total	$\chi2$	P Value
Negative	72 (97.3%)	65 (87.8%)	137 (92.6%)	4.812	0.028
Positive	2 (2.7%)	9 (12.2%)	11 (7.4%)		
Total	74 (100.0%)	74 (100.0%)	148 (100.0%)		

		Routine HPE			McNemar's test	
		Positive	Negative	Total	$\chi2$	P Value
IHC CD 138	Positive	2 (2.7%)	7 (9.5%)	9 (12.2%)	7.000	0.008
	Negative	0 (0.0%)	65 (87.8%)	65 (87.8%)		
	Total	2 (2.7%)	72 (97.3%)	74 (100.0%)		

Two samples which were diagnosed as chronic endometritis in Routine Histopathology were also positive for plasma cells thus labelled as CE in IHC for CD138 testing also. (Table 2) In 7(9.5%) additional samples which were diagnosed as normal in routine HPE, plasma cells were detected after IHC CD138 staining and thus diagnosed Positive for chronic endometritis. Hence, in total 9 patients were diagnosed with Chronic Endometritis.

The correlation was statistically compared with Chi-Square test and McNemar's test. The overall difference was statistically significant (McNemar's test: $\chi2 = 7.000$, $p = 0.008$). (Table no 3) Thus IHC CD 138 staining significantly improved the detection of chronic endometritis.

Table 4: Microbial Culture Results

Microbial Culture	Frequency (n = 75)	Percentage
No Growth	65	86.7%
Escherechia coli	3	4.0%
Streptococcus Species	3	4.0%
Staphylococcus Species	2	2.7%
Acinetobacter baumanii	1	1.3%
Staphylocccus Aureus	1	1.3%

Gram Staining and Microbial Culture for aerobic and microaerophilic organisms were done in all 75 cases. 65 samples had no growth in gram staining. Out of the rest 10 samples, 6 samples (8.0%) showed Gram Positive Cocci and 4 samples (5.3%) had showed Gram Negative Bacilli. In all these cases, organisms had growth on cultures. The microorganisms detected in culture were E. coli and Streptococcus in majority, followed by Staphylococcus and Acinetobacter (Table 4). The patients with growth in microbial cultures were given antibiotic treatment according to sensitivity. Doxycycline was the most common antibiotic to whom the Staphylococcus and Streptococcus organisms were sensitive.

Table 5: Genexpert Test Result

GeneXpert test	Frequency (n = 75)	Percentage
Positive	1	1.3%
Negative	74	98.7%

For identification of Endometrial Tuberculosis, ZN staining and GeneXpert were done. None of the samples were positive in ZN Staining. One sample was positive for mycobacterium tuberculosis on GeneXpert test (Table 5).

All the patients were planned for Ovulation Induction (OI) with Intrauterine Insemination. 16 patients were lost to follow up either due to covid situation or other personel reasons. One patient diagnosed as Tubercular endometritis is continuing Anti-Tubercular Treatment (ATT). Out of 9 patients diagnosed with CE, one patient underwent one cycle of IUI and rest 8 underwent 2 cycles of IUI. 2 patients (22.2%) in the CE group had conceived after OI with IUI. 17 patients (34.7%) in the without CE group had conceived out of which 5 patients conceived spontaneously and 12 patients after OI with IUI. Fisher's exact test was used to explore the association between the two subgroups and Conception outcome. There was no significant difference between the various groups in terms of distribution of Conception. ($\chi2 = 2.534$, $p = 0.154$).

DISCUSSION

Infertility is a concern of global importance in reproductive health. Chronic Endometritis is an emerging cause of infertility. It damages the endometrial stroma by low grade inflammatory changes thus preventing fertilization or causing recurrent miscarriage. It is quite difficult to diagnose as Chronic Endometritis remains asymptomatic and basic infertility evaluation remain normal. In this study, we tried to evaluate the prevalence of Chronic Endometritis among unexplained infertility with various methods in Indian population. In the present study, endometrial biopsy was taken in the late Follicular Phase of menstrual cycle because various studies showed higher incidence of presence of plasma cells in proliferative endometrium as compared to secretory endometrium. Also common findings in secretory endometrium like superficial edematous changes and elevated stromal cell density interfere with the identification of plasma cells and thus make it difficult to diagnose chronic endometritis.In the study conducted by Adegboyega et al[13] on diagnosing CE using histopathology, he found biopsies in the proliferative phase were much more likely to be CD138 positive (69.2%) compared with biopsies from endometrium in the late secretory or menstrual phase. In the present study, 12% of the women with unexplained infertility were diagnosed with chronic endometritis on histopathological examination with IHC CD1389 of endometrial biopsy specimen. Similar result was found in the study conducted by Liu Y et al[3] where 10.4% women of infertility and 7.7% of recurrent implantation failure were affected with CE. On the contrary, in the study conducted by Cicinelli et al[2], 56.8% of the cases were positive in hysteroscopy and also at histopathology including IHC which is much higher than our study. In another study conducted by Cicinelli et al[8] on prevalence of CE in unexplained RIF, 66% of cases diagnosed by hysteroscopy and 57% were diagnosed with CE by HPE. The results were higher than our study. In the current study, we found that all the 9 cases showed presence of plasma cells in IHC CD138, but only 2 cases were positive on routine histopathology by H&E staining. The difference between two methods was statistically significant in detecting CE. Thus the rate of detection of chronic endometritis by IHC CD138 was greater than H&E staining making it more sensitive. The difference between the two methods was statistically significant. McQueen et al[9] also in his study found that the use of CD138 IHC staining resulted in a significantly higher prevalence of CE compared with the use of H & E staining (56% [60/107] vs. 13% [14/107]).

The microorganisms detected were E. coli, Streptococcus sp. and Staphylococcus. In the study conducted by Cicinelli et al[2], among 95 unexplained infertile women included, endometrial culture was positive in 34 women where common agents found were Enterococcus faecalis, mycoplasma, E. coli, Streptococcus sp. and Staphylococcus which is comparable to our study. Similarly, in the study conducted by Inmaculada Moreno et al[10] among asymptomatic infertile women, the most abundant microorganisms that were detected by cultures and molecular method were Streptococcus species (47%), followed by Enterococcus (15%), E. coli (12%), Staphylococcus (3%).

In our study, though we tested all endometrial samples for mycobacterium tuberculosis by GeneXpert testing also along with ZN staining and HPE, we found very low detection rate of 1.3% of endometrial Tuberculosis in unexplained infertility. Contrary to our study, MD Kohli et al[11] and Danish Zahoor et al[12] in their study among infertile women in North, India found higher cases of Mycobacterium tuberculosis (19 positive out of 100 cases i.e. 1.8% and 6.73%

respectively). In the current study, we found no difference in the pregnancy rate between the CE and without CE group after antibiotic treatment. Total 19 patients conceived i.e. 32.7%, out of which, 2 patients were in the CE group and 17 in the without CE group. Other studies have found improvement in the conception rates after treatment in the CE cases. Cicinelli et al[8] found higher pregnancy rate with IVF in resolved chronic endometritis group after receiving antibiotic therapy as compared to the group with persistent chronic endometritis (65.2 versus 33%). Similarly, Cicinelli et al[2] in his another study also found higher pregnancy rate among cured chronic endometritis women compared with persistent disease and without chronic endometritis diagnosis. Hence other studies conclude that antibiotic treatment improves the pregnancy and live birth rate which is lacking in our study.

CONCLUSION

Chronic endometritis is an important and emerging cause of unexplained infertility. In our study we detected chronic endometritis on endometrial biopsy in 12.2% of the unexplained infertility cases in Indian population. Immunohistochemistry for CD138 was found to be much superior to routine histopathology in diagnosing chronic endometritis. Tubercular endometritis was detected in only one case (1.3%) in our study population.

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