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Enhancing endometrial pathology detection: chromohysteroscopy vs. conventional hysteroscopy in infertility evaluation

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Abstract

Background Endometrial pathology is a recognized contributor to infertility and recurrent implantation failure, but its detection during hysteroscopy, particularly in the absence of visible lesions, is challenging. Chromohysteroscopy, involving the use of methylene blue dye, has shown promise in improving diagnostic accuracy.

Methods This interventional cross-sectional study compared the diagnostic accuracy of chromohysteroscopy and conventional hysteroscopy, using histopathology as the gold standard. A total of 100 consenting infertility patients were included, in a sequential step, hysteroscopy followed by chromohysteroscopy using methylene blue dye was performed to enhance the detection of subtle endometrial pathologies.

Results Among the 100 women who underwent diagnostic hysteroscopy, 76 exhibited normal findings, while 24 had abnormal findings. During chromohysteroscopy, 62 cases displayed a light staining pattern, and 38 cases exhibited dark staining. Histopathological analysis of biopsy tissue from the dark-stained areas revealed endometrial pathology in 84.2% (32/38) and normal endometrium in 15.8% (6/38) cases. In contrast, biopsies from the light-stained areas indicated abnormal endometrium in only 3.23% (2/62) cases, with the remaining 96.77% having a normal histology. Chromohysteroscopy demonstrated a significantly higher diagnostic accuracy (92%) for endometrial pathology compared to conventional hysteroscopy (58%). Notably, chromohysteroscopy's sensitivity was 94.12%, and its specificity was 90.9%. The staining patterns observed during chromohysteroscopy correlated well with histological findings, highlighting its effectiveness in identifying structurally damaged endometrium.

Conclusion Chromohysteroscopy emerges as a valuable diagnostic tool for assessing endometrial pathology, especially in infertility cases. This technique offers superior accuracy compared to conventional hysteroscopy, providing clinicians with a more precise means of diagnosis and potential targeted treatments.

Keywords Chromohysteroscopy, Endometrial pathology, Methylene blue, Hysteroscopy

Background

Hysteroscopy enables the direct observation of the uterine cavity and offers opportunities for concurrent treatment of various uterine abnormalities. However, in cases where hysteroscopy reveals no obvious abnormalities and the uterus is deemed normal, there may still be underlying cellular abnormalities that are not visually apparent [1]. Hysteroscopy is recognized as a crucial tool in

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evaluating the uterine cavity [2–4], yet its ability to detect subtle endometrial changes at the cellular level, which do not manifest as macroscopic abnormalities, is limited. To address this limitation, endometrial staining with vital stains, such as 1% methylene blue during conventional hysteroscopy, can be employed to identify apoptotic endometrial cells.

Under normal circumstances, the endometrium is a non-absorptive epithelium that does not take up dye. The American Society for Reproductive Medicine has emphasized that methylene blue dye can only penetrate the cytoplasm and stain the nucleus if the cells and their membranes are damaged [5]. Minimal apoptosis is typically present in the endometrium due to the cyclical shedding during menstruation, resulting in light blue staining, which is considered normal. Conversely, dark blue staining indicates significant apoptosis due to endometritis. Targeted endometrial biopsies from these areas can be obtained for histopathological examination [6].

Chronic endometritis is identified as a contributing factor in approximately 30% of infertility cases associated with uterine factors [7]. It adversely affects successful embryo implantation, primarily due to reduced endometrial receptivity resulting from microbial effects, the presence of specific lymphocyte subtypes in the endometrium creating an abnormal microenvironment that hinders endometrial receptivity, local immune responses, and an unfavorable environment in the endometrium for recruiting circulating B cells [8].

Recent meta-analyses on the effects of chronic endometritis therapy on in vitro fertilization outcomes have demonstrated that treating endometritis leads to improved implantation rates [7]. Detecting and treating endometritis early can result in better reproductive outcomes for affected women. Only a few studies have explored the role of chromohysteroscopy in diagnosing endometrial pathology in infertile patients. This study aims to assess the effectiveness of chromohysteroscopy using methylene blue dye in detecting endometrial pathologies in infertility patients.

Materials and methods

This interventional cross-sectional study received approval from our institution's ethical committee (AIIMS/Pat/IEC/2020/449). We included all patients who visited AIIMS, Patna, and were diagnosed with infertility during a 24-month period, spanning from July 2021 to June 2023. These patients provided their consent to participate in the study. Excluded from the study were individuals with evident gross endometrial abnormalities detected during hysteroscopy, such as fibroids, polyps, congenital uterine anomalies, endometrial hyperplasia, and endometrial malignancy. Also excluded were patients with a current or past history of tuberculosis treatment, those previously diagnosed with tubercular endometritis through endometrial biopsy, individuals with active pelvic inflammatory disease (PID), and patients with diagnosed bleeding disorders.

Following the assessment of pre-anesthetic fitness, patients underwent hysteroscopy during the postmenstrual phase to directly visualize the uterine cavity and uterine lining. In cases where it was deemed necessary, laparoscopy with chromopertubation was performed. The choice of anesthesia (conscious sedation, short general anesthesia, or spinal anesthesia) was based on the expected duration of the procedure and the clinical characteristics of the patients in the operating room.

All hysteroscopies were carried out using a vaginoscopy approach by a single surgeon employing a 2.9-mm hysteroscope. Uterine distension was achieved using a continuous flow and pressure-controlled pump system with normal saline. The hysteroscopy followed the standard protocol, involving the inspection of the cervix, endocervical canal, uterine cavity, tubal ostia, and endometrium. The presence of any obvious pathology was noted. Following this, patients underwent chromohysteroscopy-guided endometrial biopsy. In chromohysteroscopy, a 10-ml solution of 2% methylene blue dye was instilled into the uterine cavity using a sterile disposable 20-mL plastic syringe connected to the inflow port of the hysteroscope to stain the endometrium. Five minutes after dye instillation, the normal saline distending medium flow was restarted to wash the endometrium. Uterine distension with normal saline was maintained for one minute to evenly distribute and flush the dye. The staining pattern of the endometrium was then observed and categorized for statistical analysis. The staining patterns were classified as follows: no staining (Fig. 1A); diffuse light blue staining, considered normal; dark focal or diffuse blue staining above the cervical ostium, regardless of the size and number of stained areas, was regarded as a positive finding (Fig. 1B).

Biopsies were obtained from both dark and light-stained areas guided by hysteroscopy. Subsequently, endometrial biopsies were performed blindly using Karman's cannula. The collected endometrial samples were labeled and fixed in separate containers with 10% formalin for histopathological examination using hematoxylin and eosin staining. All findings from the blind endometrial biopsies and chromohysteroscopy-guided biopsies were documented. Following hysteroscopy, all patients were discharged after an 8-h observation period.

Statistical analysis

Quantitative data was shown as the mean ± standard deviation (SD) or median with interquartile range

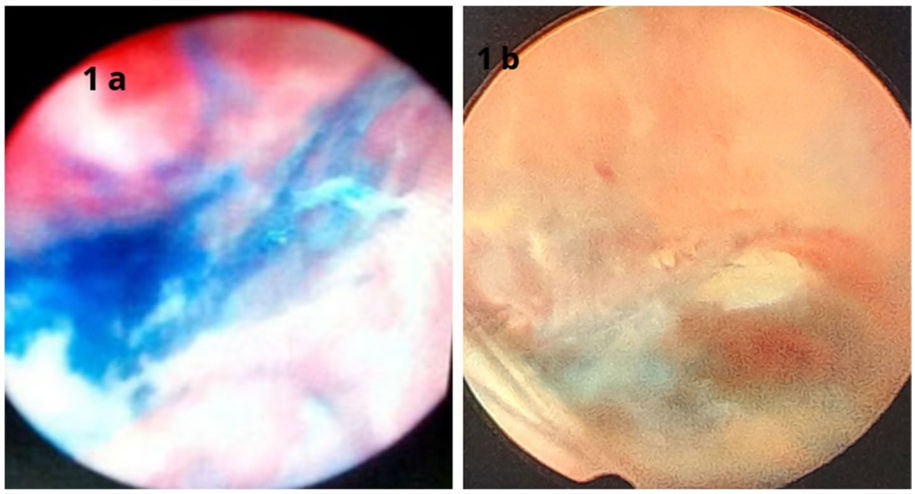

Fig. 1 **A** Focal dark staining of damaged endometrium with interspersed unstained areas on staining with methylene blue dye. **B** Light-stained endometrium seen on chromohysteroscopy

(IQR). Qualitative variables were expressed as absolute and relative frequencies. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of chromohysteroscopy and hysteroscopy were calculated keeping the histopathology examination report as a gold standard. The chi-square test was used to find the association of endometrial pathologies with other qualitative variables. Data was given a 95% confidence interval. *P* value < 0.05 was considered significant. Statistical analysis was performed by MedCalc Statistical software version 19.2.6 (medcalc.org; 2021).

Results

In this study spanning 24 months, approximately 400 infertility patients visited the outpatient department. Out of these, 160 patients met the inclusion criteria, and 100 of them provided consent to participate.

The average age of the cases was 28 years (as indicated in Table 1). Among the studied population, 62% were nulliparous, while 12% were multiparous. Additionally, 59 women had less than 3 years of infertility, while the remaining 41 had been experiencing infertility for more than 3 years. Of the total, 62 women had primary infertility, and 38 had secondary infertility. Fifty-eight women received general anesthesia, 38 received conscious sedation, and 4 received spinal anaesthesia.

Table 2 presents the hysteroscopy findings, staining pattern distribution, and their correlation with histopathology among the study participants. Hysteroscopy revealed normal findings in 76 women, whereas 24 women had abnormal findings. Among those with normal hysteroscopic findings, 34.2% (26/76) had abnormal histology, while 65.7% (50/76) had normal histology reports. Among those with abnormal hysteroscopic findings, 16 (66.67%) had normal histology reports and only 8 (33.34%) had abnormal histology. During chromohysteroscopy, 62% of the endometrium exhibited diffuse light staining, while 38% showed dark staining. Out of the 38 darkly stained areas, 84.6% (32/38) had abnormal histology, in contrast to only 3.23% (2/62) from the light-stained areas. This difference was statistically significant with a *p* value of < 0.023.

Table 3 provides details about the histopathology findings from darkly stained areas. The most common histological diagnosis was tuberculous endometritis (12/34), followed by chronic endometritis (8/34). Biopsies from darkly stained areas detected a significantly higher number of pathologies compared to unstained areas (84.2% vs. 36.85% with a *p* value < 0.003).

Table 1 Demographic details of the studied population

Demographic factors		No of cases	% of cases
Age range	< 30 years	70	70%
	> 30 years	30	30%
Mean age	28 years		
Parity	< 1	62	62%
	1–2	26	26%
	> 2	12	12%
Duration of infertility	< 3 years	59	59%
	> 3 years	41	41%
Type of infertility	Primary	62	62%
	Secondary	38	38%

Table 2 Hysteroscopy findings, staining pattern, and their HPE correlation among the studied population

HPE (*N* = 100)	Hysteroscopy (*N* = 100)		*p* value	Chromohysteroscopy (*N* = 100)		*p* value
	Normal (*N* = 76)	Abnormal (*N* = 24)		Light staining (*N* = 62)	Dark staining (*N* = 38)	
Normal endometrium (*N* = 66)	50 (65.78%)	16 (66.67%)		60 (96.77%)	6 (15.8%)	
Endometrial pathology (*N* = 34)	26 (34.21%)	8 (33.34%)	0.91	2 (3.23%)	32 (84.2%)	0.023

Table 3 Histopathology from stained, unstained, and blind biopsy in focal dark stained group (*N* = 38)

Column	HPE	Stained	Unstained	Blind biopsy
Normal	Proliferative EM	2	12	13
	Secretory EM	4	17	17
Percentage		15.8%	76.31%	78.94%
Abnormal	Disordered proliferation	4	2	2
	TB endometritis	12	5	3
	Hyperplasia without atypia	8	2	2
	Chronic endometritis	8	0	1
Percentage		84.2%	23.68%	21.05%
Total		38	38	38

Table 4 compares the diagnostic performance of hysteroscopy and chromohysteroscopy, using histopathology as the gold standard. Chromohysteroscopy demonstrated superior diagnostic capabilities, with a sensitivity of 94.12% compared to hysteroscopy's 23.53% and a specificity of 90.9% compared to hysteroscopy's 75.76%. The diagnostic accuracy of chromohysteroscopy was 92%, significantly higher than the 58% accuracy of hysteroscopy.

Discussions

Endometrial pathology is a well-recognized contributor to infertility and recurrent implantation failure, with reported prevalence rates exceeding 30% [7]. Despite the simplicity and safety of hysteroscopy in evaluating the uterine cavity, its ability to detect endometrial pathology in the absence of intracavitary lesions is limited. To enhance the detection rate of subtle endometrial pathology after diagnostic hysteroscopy, we employed the use of methylene blue dye, a substance commonly used in gastrointestinal imaging [9]. The rationale behind this was the postulation that damaged cells would allow the passage of methylene blue dye.

Our study demonstrated a significantly higher diagnostic accuracy for chromohysteroscopy compared to conventional hysteroscopy (92% vs. 58%) in the detection of endometrial pathology.

The mean age of the study population was 28 years, with the majority falling within the 26–30 years age range. This relatively young age can be attributed to early marriages, which consequently contribute to the years of infertility. These findings align with previous studies on chromohysteroscopy, which also reported mean ages of 27–29 years [10, 11].

In our study, 76% of women had normal hysteroscopic findings, while 24% had abnormal findings. Interestingly, among the 76 women with normal hysteroscopy, 34.2% had abnormal histological results. Conversely, 96.77% of cases (60/62) with diffuse light staining areas had normal histological findings. This highlights the high specificity of chromohysteroscopy in detecting endometrial pathology, a finding consistent with other studies, including Kucuk et al. [11].

Furthermore, among the 34 cases with abnormal endometrial histology, 32 exhibited dark staining during chromohysteroscopy, indicating a strong correlation between staining patterns and endometrial pathology. Similar findings were reported in studies by Vijay et al. [12] and Mansour et al., [13] emphasizing the usefulness

Table 4 Diagnostic ability of hysteroscopy and chromohysteroscopy in detecting endometrial pathology

Methods of diagnosis	Endometrial pathology (*N* = 34)	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy	+LR
Hysteroscopy		23.53%	75.76%	29.4%	69.8%	58%	0.97
Normal (*N* = 76)	26 (34.21%)						
Abnormal (*N* = 24)	8 (33.34%)						
Chromohysteroscopy		94.12%	90.9%	81.6%	97.3%	92%	10.3
Light staining (*N* = 62)	2 (3.23%)						
Dark staining (*N* = 38)	32 (84.2%)						

of chromohysteroscopy in diagnosing subtle endometrial changes.

Comparing histopathology results between the dark-stained and unstained areas within the dark-stained group ($N=38$), the power of dark staining to detect endometrial pathology was statistically significant ($p=0.003$). This aligns with studies by Haider et al. wherein they demonstrated a sensitivity of 83.3% with a diagnostic accuracy of 82.8% of dark staining area in detecting endometrial pathology, and they concluded that the difference in the diagnostic ability of stained tissue biopsy was highly significant ($p<0.001$) when compared to blind endometrial aspiration biopsy [14]. Singh et al. also demonstrated the superiority of stained tissue biopsy over unstained tissue biopsy in diagnosing endometrial pathology [15]. In the current study, the most frequently observed histological condition was tuberculous endometritis, followed by chronic endometritis and endometrial hyperplasia. Conversely, in a study conducted by Kucuk et al., which involved 64 women experiencing IVF failure, chronic endometritis was reported as the most common endometrial pathology. This disparity in findings could potentially be attributed to the high prevalence of genital tuberculosis in our country.

In the current study, chromohysteroscopy demonstrated a diagnostic accuracy of 92% for detecting endometrial pathology, in contrast to the 58% accuracy observed with simple hysteroscopy. These findings align with a previous study conducted by Gupta et al., which reported a diagnostic accuracy of 86.67% for chromohysteroscopy in the evaluation of endometrial pathology. These findings suggest that this approach can significantly improve the accuracy of diagnosis and guide clinicians in providing more targeted and effective treatments for patients experiencing fertility challenges.

The endometrium does not function as an absorptive epithelium, but structural damage to cells during apoptosis can permit the entry of methylene blue dye into the cells. In this study, the presence of dark blue staining indicates significant apoptotic areas caused by endometritis. Affected areas of the endometrium exhibit reduced responsiveness to steroid hormones. It has been demonstrated that localized endometrial abnormalities can result in miscarriage. Eliminating these local endometrial defects may encourage the growth of healthy cells and restore receptivity, ultimately leading to successful implantation.

Strength and limitations

This study has notable strengths. Firstly, it directly compares the diagnostic accuracy of hysteroscopy and chromohysteroscopy, using histopathology as the gold standard. Secondly, it convincingly demonstrates the enhanced effectiveness of chromohysteroscopy in identifying subtle endometrial pathologies when compared to conventional hysteroscopy.

However, there are certain limitations to this study. We did not conduct patient follow-up to assess whether the pathologies identified through chromohysteroscopy were subsequently treated. Additionally, further research on a larger scale with a broader sample size is necessary to refine and improve the accuracy of this technique.

Conclusion

In conclusion, this study has shed light on the significance of chromohysteroscopy as a valuable diagnostic tool in the assessment of endometrial pathology, particularly in cases of infertility. By introducing methylene blue dye during hysteroscopy, we were able to substantially enhance the detection rate of endometrial pathology. Our findings revealed a remarkable improvement in diagnostic accuracy (92% vs 58%) with chromohysteroscopy, which outperformed conventional hysteroscopy.

The study also highlighted the correlation between staining patterns and histological findings, further emphasizing the utility of chromohysteroscopy in identifying structurally damaged areas of the endometrium, such as those affected by chronic endometritis or other pathologies.

Based on these results, we strongly recommend the routine application of chromohysteroscopy as a standard diagnostic procedure in the evaluation of endometrial disease, particularly in the context of infertility.

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Authors' contributions

Data collection and analysis were done by JBS and S. Concept, designing, and writing of the manuscript were done by SJ and S. Supervision and literature review were done by SJ. All the authors have approved the final version of the manuscript.

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Availability of data and materials

The anonymized data collected for this study will be available on reasonable request to the authors via online link https://docs.google.com/spreadsheets/d/1SORS2n6ERPl3U5A2CfzO6x4UcjCGakGE/edit?usp=drive_link&ouid=113426274015947353420&rtpof=true&sd=true.

Declarations

Ethics approval and consent to participate

The study had been approved by the institute ethical committee. (AIIMS/Pat/IEC/2020/449). Written consent had been obtained from the study participants.

Consent for publication

Written consent for publication has been obtained from the participants.

Competing interests
The authors declare that they have no competing interests.

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