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ORIGINAL ARTICLE

Role of Chromohysteroscopy in Evaluation of Endometrial Pathology Using Methylene Blue Dye

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Abstract

Background Chromohysteroscopy is expected to help in diagnosing subtle endometrial pathologies which could be missed on conventional hysteroscopy and also help in targeting biopsy from endometrium.

Objective To study staining pattern of endometrium in patients undergoing chromohysteroscopy and to evaluate and compare the histopathology of chromohysteroscopy-guided endometrial biopsy with conventional endometrial sampling.

Method This was a cross-sectional study conducted during the period of 18 months in Department of Obstetrics and Gynaecology, ESI PGIMSR, New Delhi, India, from September 2016 to February 2018. Totally, 60 women with complaints of infertility, failed intrauterine insemination (IUI), recurrent spontaneous abortions (RSA), heavy menstrual bleeding (HMB), intermenstrual bleeding (IMB) and postmenopausal bleeding (PMB) meeting inclusion criteria were evaluated and enrolled in the study. In patients with normal looking endometrium on hysteroscopy, methylene blue dye was administered through the hysteroscopic inlet. Tissue samples were obtained from stained areas followed by blind endometrial sampling immediately. The results of chromohysteroscopy-guided biopsy from light- and dark-blue-stained areas and blind biopsy were compared.

Results Mean age of the study group was 37 years, with mean BMI of 24 kg/m2. There were 24 cases of HMB, 9 of IMB, 7 of PMB, 15 of infertility, 2 of failed IUI and 3 with RSA. On chromohysteroscopy, 39(65%) cases showed light-stained endometrium (group I) and 21(35%) showed dark-stained endometrium (group II). Comparison was done between histopathology obtained through chromohysteroscopy and blind endometrial sampling. The diagnostic accuracy of chromohysteroscopy-guided endometrial biopsy in evaluation of endometrial pathology was 86.67% with sensitivity of 91.67%, specificity of 85.41%, PPV of 61.12% and NPV of 97.61% (P <0.001).

Conclusion Chromohysteroscopy was able to detect endometrial pathology which was missed on conventional hysteroscopy and detected more cases of endometrial pathology than blind endometrial sampling.

Keywords Chromohysteroscopy · Chromohysteroscopy-guided endometrial biopsy · Blind · Endometrial sampling · Dark staining · Light staining

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Introduction

Endometrial pathologies are responsible for common and varied gynaecological problem in women. The patient may present with abnormal uterine bleeding, postmenopausal bleeding, infertility, recurrent spontaneous pregnancy loss, failed intrauterine conceptions, failed in vitro implantations. Hysteroscopy is recommended for direct and closer look of the endometrium [1]. It is the best technique for identifying macroscopic endometrial pathologies like polyps, adhesions and submucous myomas that can be detected and appropriately treated. A normal appearance in conventional hysteroscopy does not assure integrity of the endometrium. Chromohysteroscopy is endometrial dying using methylene

blue dye during conventional hysteroscopy. Uterine cavity if appears to be normal on visualization on conventional hysteroscopy is stained to look for any endometrial abnormality. Patients are classified according to the staining pattern. Diffuse light blue staining is considered normal. Focal or diffuse dark blue staining above the internal cervical ostium regardless of size and number of stained areas is considered positive finding. Final histopathology report determined the endometrial pathology. In this study, we tried to find out the role of chromohysteroscopy as an adjunct to conventional hysteroscopy in the diagnosis of uterine pathology.

Methodology

This was a cross-sectional study conducted during the period of 18 months in the Department of Obstetrics and Gynaecology, ESI PGIMSR, New Delhi, India, from September 2016 to February 2018. Ethical clearance was taken from institution ethical committee. In our study, 60 women with complaints of infertility, failed IUI, recurrent spontaneous abortions, heavy menstrual bleeding, intermenstrual bleeding and postmenopausal bleeding meeting inclusion criteria were evaluated and counselled and enrolled for conventional hysteroscopy. Hysteroscopy was done in postmenstrual phase of menstrual cycle. If no gross pathology was visible on conventional hysteroscopy, patients were subjected to chromohysteroscopy-guided endometrial biopsy.

With patients under general anaesthesia, fully assembled hysteroscope (Stryker) of 6.5 mm with viewing angle of 30° was attached to the fibreoptic light source, distending medium (0.9% sodium chloride solution) and video endocamera introduced into the cervical os with the irrigating system turned on. Adequate focusing of the image was done prior to insertion of the hysteroscope which was advanced into the uterine cavity under direct vision. The uterine cavity was observed panoramically; if the endometrium was normal looking without any obvious lesion, then patient was subjected to chromohysteroscopy.

In chromohysteroscopy, 10 ml of 2% solution of methylene blue dye was instilled into the uterine cavity using a disposable sterile 20-mL plastic syringe connected to the inflow port of the hysteroscope to stain the endometrium. Five minutes following the injection of dye, the distending medium flow was started again to wash the endometrium. Uterine distension with normal saline then resumed for one whole minute, in order to distribute and flush the dye. The staining pattern of the endometrium then noted and allocated, for statistical convenience, to be one of either, no staining, dark focal/diffuse staining of the endometrium, light diffuse staining of the endometrium. Dark focal/diffuse blue staining above the cervical ostium, regardless of size and number of stained areas, was considered positive finding. Tissue samples were obtained from stained (dark-stained or light-stained) areas under hysteroscopic guidance. Hysteroscope was then taken outside, and blind endometrial biopsies were taken and sent for histopathological examination. The collected data were entered in an MS Excel spreadsheet systematically. The data analysis was done by using the statistical package for the social science system version SPSS 17.0.

Results

Totally, 60 cases were included in the study. Out of them, 24 cases had complaint of heavy menstrual bleeding, 9 had intermenstrual bleeding, 7 had postmenopausal bleeding, 15 cases were of infertility, 3 had recurrent spontaneous abortions, and 2 had failed IUI. The mean age of the cases was 37 years. Majority of the cases were of normal weight with mean BMI of 24 kg/m^2. Sixty-five per cent cases had endometrial thickness between 4.0 and 7.9 mm. Eleven cases (18%) had ET > 12 mm of which 7 complained of HMB and 3 complained of IMB. Forty-two per cent cases which had ET > 12 mm showed dark-stained endometrium, while 79.4% cases which had ET between 4.0 and 7.9 mm showed light-stained pattern on chromohysteroscopy. Correlation was seen between ET and complaints ($p = 0.042$) and was statistically significant. After chromohysteroscopy staining, pattern of the endometrium was noted. It was grouped as 'light-stained endometrium' (group I) and 'dark-stained endometrium' (group II). Dark staining (group II) was further subdivided into 'dark focal' (group IIa) and 'dark diffuse' (group IIb). Thirty-nine (65%) cases showed light-stained endometrium (group I), and 21 (35%) cases showed dark-stained endometrium (group II) (Figs. 1 and 2).

On chromohysteroscopy-guided endometrial biopsy (CHPE), 36 cases were reported as secretory endometrium,

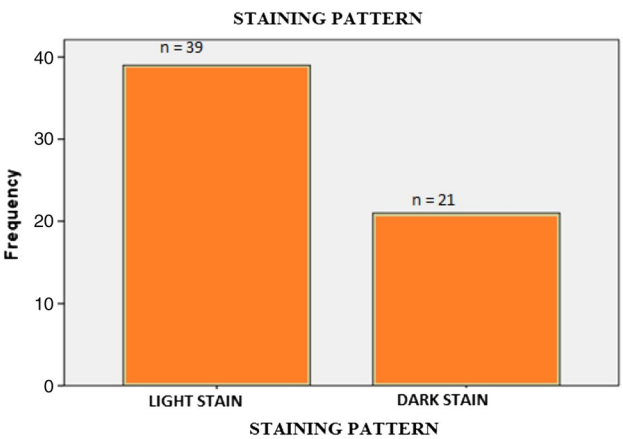

Fig. 1 Staining pattern on chromohysteroscopy and distribution according to light ($n = 39$) and dark ($n = 21$) staining

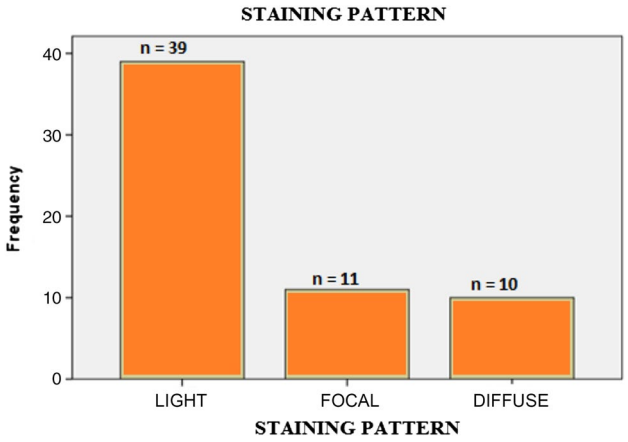

Fig. 2 Distribution between light staining ($n=39$), dark focal staining ($n=11$), dark diffuse staining ($n=10$)

while on blind endometrial sampling (BES) 42 cases reported as secretory endometrium. Hence, BES missed 6 cases of endometrial pathology and reported as secretory. Both CHPE and BES detected equal number of proliferative endometria ($n=6$). CHPE detected 11 cases of endometritis as compared to BES which detected only 7 cases. This suggests that BES reported 4 cases of endometritis as secretory endometrium. One case of dark focal staining was reported as secretory endometrium on CHPE which was reported endometritis on BES. Four cases of hyperplasia without atypia were picked up by CHPE and 3 cases picked up by BES. Therefore, 1 case was missed by BES and reported as normal endometrium (secretory). Two cases of hyperplasia with atypia were picked up by CHPE, but BES picked 1 case only. Detection of endometrial cancer was same by both CHPE and BES. Both showed 1 case only. Dark-staining CHPE detected 16 cases of endometrial pathology (9 endometritis, 4 hyperplasia without atypia, 2 hyperplasia with atypia and 1 endometrial carcinoma). Light-staining CHPE detected 2 cases (both endometritis) of endometrial pathology. Blind endometrial sampling (BES) detected 12 cases of endometrial pathology (7 endometritis, 3 hyperplasia without atypia, 1 hyperplasia with atypia and 1 endometrial carcinoma). Single case of endometrial carcinoma was detected by both chromohysteroscopy and blind endometrial sampling (Table 1).

CHPE detected 18 cases of endometrial pathology, while BES detected 12 cases of endometrial pathology and missed 6 cases (Table 2).

Thus, the diagnostic accuracy of chromohysteroscopy in evaluation of endometrial pathology was 86.67% with sensitivity of 11/12 = 91.67%, specificity of 41/48 = 85.41%, PPV of 11/18 = 61.11% and NPV of 41/42 = 97.61%. In this study, 45.8% (11/24) of HMB, 18% (2/11) of IMB, 14% (1/7) of PMB, 26.7% (4/15) of infertility, 33% (1/3) of RSA and 100% (2/2) cases of failed IUI showed dark-stained endometrium on chromohysteroscopy. No correlation was seen between complaints and the staining pattern ($p=0.092$). After noticing the staining pattern, biopsy was taken from stained areas and sent for HPE in all the patients. 76.1% (16/21) cases from group II (dark-stained) had 'endometrial pathology' on HPE. Focal dark staining detected endometrial pathology in 7/11 cases, (63.6%) and dark diffuse staining detected 9/10 (90%) cases. In this study, dark focal staining was mostly seen in cases of endometritis (5/11 = 45%) and dark diffuse staining was mostly seen in cases hyperplasia with or without atypia and endometrial carcinoma (5/10 = 50%) (Figs. 3, 4, 5) In cases of group I, light-stained endometrium, 94.8% (37/39) showed normal endometrium and 5.2% showed endometrial pathology (2/39) (Fig. 2). Thus, it is emphasized that dark-staining pattern was mostly seen in cases of endometrial pathology and light staining was seen in cases of normal endometrium. Blind endometrial sampling (BES) was followed after

Table 1 Comparison between the histopathology obtained from chromohysteroscopy (CHPE) and blind endometrial sampling (BES)

	Chromohysteroscopy vs. blind endometrial sampling						
	Blind endometrial sampling						Total
	Secretory endometrium	Proliferative endometrium	Endometritis	Hyperplasia without atypia	Hyperplasia with atypia	Endometrial cancer	
Chromohysteroscopy							
Secretory endometrium	35	0	1	0	0	0	36
Proliferative endometrium	0	6	0	0	0	0	6
Endometritis	5	0	6	0	0	0	11
Hyperplasia without atypia	1	0	0	3	0	0	4
Hyperplasia with atypia	1	0	0	0	1	0	2
Endometrial Cancer	0	0	0	0	0	1	1
Total	42	6	7	3	1	1	60

Table 2 Correlation between histopathology obtained by chromohysteroscopy and blind endometrial sampling

		BES		Total
		Endometrial pathology	Normal	
CHPE	Endometrial pathology	11	7	18
	Normal	01	41	42
Total		12	48	60

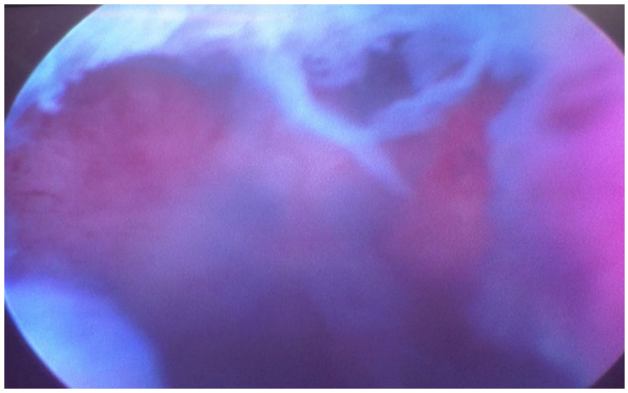

Fig. 3 Dark focal staining pattern on chromohysteroscopy

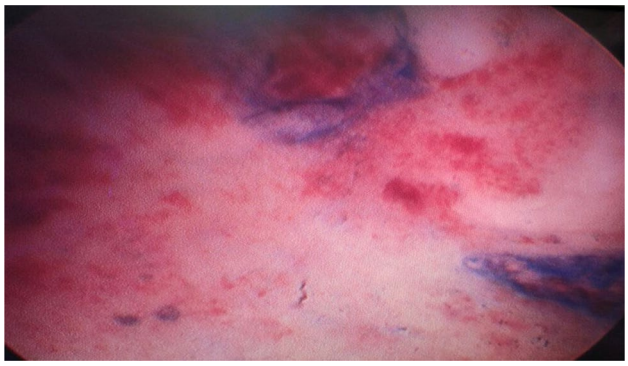

Fig. 4 Light-staining pattern on chromohysteroscopy

Fig. 5 Biopsy being taken from dark-stained area during chromohysteroscopy

chromohysteroscopy for all the patients. Both the biopsies (CHPE and BES) were sent for HPE. BES reported 48 cases (80%) as normal endometrium while 12(20%) cases as endometrial pathology. Chromohysteroscopy detected pathology in 76.1% cases (16/21). Thus, BES failed to detect endometrial pathology which was picked up on chromohysteroscopy (2 cases of endometrial pathology were picked up by BES which were missed on CHPE).

In our study, there were total 40 cases of AUB which included 24 cases of HMB (60%), 9 cases of IMB (22.5%) and 7 cases of PMB (17.5). In cases of HMB ($n=24$), dark staining was seen in 11 (45.8%). Dark diffuse staining was seen in more number of cases (7/11) than dark focal (4/11). Dark-stained area biopsy diagnosed 3 cases of endometritis (27%), 4 cases of endometrial hyperplasia without atypia (36%), 2 cases of endometrial hyperplasia with atypia (18.1%) and 1 case of endometrial carcinoma (9%). In light-stained biopsy (13) all had normal endometrium (100%) on histopathology.

In cases of IMB (n = 9), 2 had dark diffuse staining (22%) and endometritis was seen in 22% cases (2/9). One was missed by chromohysteroscopy which was although dark-stained and picked up by BES. In PMB ($n=7$), only one case had dark staining (14%) and none had any endometrial pathology on biopsy.

In the study, 15 patients with complaints of infertility were enrolled. Four of them had dark staining on endometrium (33.3%), and among these 4 cases 3 had endometritis on HPE report (75%). There were only two cases of failed intrauterine insemination. Both the cases had 'dark focal staining' on chromohysteroscopy. Biopsy from the stained areas had secretory endometrium on HPE in both cases, but on blind endometrial sampling 1 out of 2 cases had endometritis (50%).

This study highlights that more cases endometrial pathology can be picked up by chromohysteroscopy. The diagnostic accuracy of chromohysteroscopy-guided endometrial biopsy in evaluation of endometrial pathology was 86.67% with sensitivity of 91.67%, specificity of 85.41%, PPV of 61.12% and NPV of 97.61% ($P<0.001$). After chromohysteroscopy, 23.3% (14/60) cases did not experience any pain and 71.7% (43/60) experienced mild pain which was managed by analgesics. None of the patients experienced any complications. The mean duration for the hysteroscopy procedure was 22 min, and average of 650 ml of normal saline (distension media) was used in chromohysteroscopy.

Discussion

Yahia et al. [2] in 2014 used chromohysteroscopy in 50 postmenopausal women which led to diagnosis of three more cases of endometritis, two more cases of endometrial hyperplasia but none of endometrial carcinoma as compared to standard blind fractional curettage. In our study out of 7 cases with PMB, 14% (1/7) showed dark staining on chromohysteroscopy with no detection of endometrial pathology on histopathology. This might be due to less number of PMB cases in our study.

Singh and Singh [3] enrolled 60 patients of abnormal uterine bleeding. Out of 60 cases, 11 cases were found to have non-hormonal pathology after chromohysteroscopic biopsy. Eight (72.72%) cases were diagnosed by stained endometrial tissue, one (9.09%) by unstained tissue, and three (27.27%) by endometrial aspirations. The diagnostic ability of the stained tissue was significantly higher ($p = 0.005$) than unstained biopsy and endometrial aspirations. In 2016, Singh et al. [4] performed another study in 50 cases of abnormal uterine bleeding, but this time using a different dye, i.e. toluidine blue in place of methylene blue. In this study, they included the patients with intracavitary lesions like endometrial polyp, submucosal fibroid, ulcerative lesions, etc. Twenty-four per cent cases showed endometrial hyperplasia/carcinoma, 75% cases of endometrial hyperplasia/carcinoma showed > 50% staining of endometrial surface staining, while only 52% of cases with normal HPE showed similar staining, but the difference was not statistically significant. The sensitivity, NPV, diagnostic accuracy of stained biopsy (83.3%, 95% & 96%), unstained biopsy (83.3%, 95%, 96%) and endometrial aspiration (75%, 92.6%, 94%) did not show any statistical difference.

Alay et al. [5] in 2014 enrolled 38 patients with abnormal uterine bleeding, and chromohysteroscopy with methylene blue dye was done. In their study from the same patient, two biopsies were taken, one from stained site and another from unstained site, and then endometrial aspiration was done at the end from the same patients. Six cases of endometrial pathology were detected from stained biopsy, while 7 detected from unstained areas. Blind endometrial sampling also detected 7 cases of endometrial pathology. According to them, there was no statistical difference between the stained, unstained and BES. The insignificance of the result was maybe because stained and unstained biopsy were taken from the same patient, while in our study we took biopsy from dark-stained and light-stained areas from different patients and then compared with blind endometrial sampling. In our study there were total 40 cases of AUB which included 24 cases of HMB (60%), 9 cases of IMB (22.5%)&7 cases of PMB (17.5%)

In cases of HMB ($n = 24$), dark staining was seen in 11 cases out of 24 (45.8%). Dark diffuse staining was seen in more number of cases (7/11) than dark focal staining (4/11). Dark-stained area biopsy diagnosed 3 cases of endometritis (27%), 4 cases of endometrial hyperplasia without atypia (36%), 2 cases of endometrial hyperplasia with atypia (18.1%) and 1 case of endometrial carcinoma (9%). In the light-stained biopsy (13), all had normal endometrium (100%) on histopathology.

In cases of IMB ($n = 9$), 2 had dark diffuse staining (22%) and endometritis was seen in 22% cases (2/9). One was missed by chromohysteroscopy which was although dark-stained and was picked up by BES.

In PMB ($n = 7$), only one case had dark staining (14%) and none had any endometrial pathology on biopsy.

In the study (2017) by Chopra at al. [6] in women with unexplained infertility to find out the diagnostic accuracy of chromohysteroscopy, light-staining pattern was seen in 56 cases, and 44 cases had dark staining. Histopathology of biopsy tissue from these dark-stained areas showed endometritis in 50% (22 out of 44 cases) and normal endometrium in 50% (22 out of 44) cases, while biopsy from light-stained area showed chronic endometritis in 5.35% (3 out of 56) cases and remaining 94.65% had normal endometrium. Diagnostic accuracy of chromohysteroscopy was the following: sensitivity = 88%, specificity = 70.66%, PPV = 50%, NPV = 94.6%. They concluded that chromohysteroscopy is a simple and effective technique for diagnosing endometrial pathology in cases of infertility.

In our study, 15 patients with complaints of infertility were enrolled. Four of them had dark staining on endometrium (33.3%), and among these 4 cases 3 (75%) had endometritis on HPE report.

Hysteroscopy has been considered to be well tolerated. In our study, 14 patients (23%) did not perceive any pain postoperatively, 43 (71.7%) patients perceive mild pain which was managed easily with analgesics, and only 3 patients complained of moderate pain postoperatively. In 2014, Teran-Alonso et al. [7] showed that only 6% of the patients perceived non-pain side effects like nausea and vomiting.

Conclusion

Chromohysteroscopy was able to detect endometrial pathology which was missed on conventional hysteroscopy and detected more cases of endometrial pathology than blind endometrial sampling. This study showed that light-stained areas on endometrium were highly suggestive of normal endometrium and presence of dark-stained endometrium (focal or diffuse) was highly suggestive of endometrial pathology which should be confirmed on histopathology. It

can be used to target biopsy site during hysteroscopy which can yield better results on histopathology. Chromohysteroscopy adds to the diagnostic accuracy of conventional hysteroscopy in evaluation of endometrial pathologies. This study emphasizes that more cases endometrial pathology can be picked up by chromohysteroscopy.

Compliance with ethical standards

Conflict of interest We, Taru Gupta, Sonam Singh and Anand Kumar Verma, are submitting a manuscript titled 'Role of chromohysteroscopy in evaluation of endometrial pathology using methylene blue dye', and we are responsible for disclosing all financial and personal relationships that might bias their work. We state that there is no potential conflict of interest. We also declare that this study was conducted in a postgraduate institute and no funding was involved. We, Taru Gupta, Sonam Singh and Anand Kumar Verma, declare that we have no conflict of interest.

Ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national). Informed consent was obtained from all the patients for being included in the study. An ethical clearance has also been taken from the institutional ethical committee.

Informed Consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 [5]. Informed consent was obtained from all patients for being included in the study. An ethical clearance has also been taken from the institutional ethical committee.

Human and animal rights This article does not contain any studies with animal subjects.

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