

Role of Chromo-hysteroscopy with Toluidine Blue in Diagnosing Endometrial Pathology after 40 years of Age

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Abstract

Objective: To evaluate diagnostic accuracy of Chromo hysteroscopy with Toluidine Blue for endometrial pathology in women with AUB after 40 years of age.

Material and methods: This cross-sectional interventional study was conducted in 50 cases with menorrhagia, metrorrhagia, polymenorrhagia or postmenopausal bleeding. After excluding thyroid disorders, coagulation disorders and cervical & vaginal causes, diagnostic hysteroscopy was done and findings recorded. Chromohysteroscopy was done by instillation of 1% toluidine blue dye. Hysteroscopic biopsy was taken from stained area & unstained area separately followed by endometrial aspiration biopsy. The histopathological results of these three samples were compared for each participant.

Results: The ability of hysteroscopy to diagnose endometrial pathology was significantly higher ($p=0.013$) in comparison to TVS. 24% cases showed endometrial hyperplasia/carcinoma. 75% cases of endometrial hyperplasia/carcinoma showed $\geq 50\%$ endometrial surface staining while only 52.63% of cases with normal HPE showed similar staining but the difference was not statistically significant. The sensitivity, NPV and 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 statistically significant difference.

Conclusions: Toluidine blue dye does not specifically stain endometrial hyperplasia or endometrial carcinoma and hence is NOT a useful stain for Chromo hysteroscopy guided biopsy in diagnostic evaluation of cases of AUB.

Keywords Abnormal Uterine bleeding (AUB); Toluidine blue dye; Endometrial cancer; Endometrial hyperplasia; Hysteroscopy; Endometrial biopsy

Introduction

Endometrial Cancer is the sixth most common cancer in females worldwide. The lifetime risk of developing the disease is approximately 2.7%. Endometrial cancer often develops in the setting of endometrial hyperplasia that is now being established as a premalignant state. Clinical presentation of both endometrial hyperplasia and endometrial carcinoma is similar. They present as menorrhagia, poly-menorrhoea, inter-menstrual bleeding or postmenopausal bleeding. Almost 15% of outpatient cases and 25% of gynecologic surgery cases present with abnormal uterine bleeding (AUB) [1].

Such abnormal uterine bleeding (AUB) in perimenopausal & menopausal women is routinely investigated with ultrasonography, hysteroscopy and endometrial biopsy. Hysteroscopy guided endometrial biopsy is considered gold standard since it allows direct visualization of the endometrial lesions and directed biopsy thereafter. Sometimes focal endometrial hyperplasia or early endometrial cancer may also be missed on hysteroscopy. Chromo-hysteroscopy is a new technique in which some staining agent is used during hysteroscopy to identify focal endometrial lesions. Chromo hysteroscopy identifies endometrium to be targeted for biopsy with the help of staining dyes like methylene blue, Lugol's solution, Congo red and Indigo Carmine

[2]. The earliest use of Methylene blue or other vital stains or dyes in the endometrium dates back to 1980s when it was used to distinguish normal endometrial tissue from fibrotic one in Asherman's syndrome [3]. Ozturk et al., conducted a cross-sectional study in 50 patients to evaluate the usefulness of endometrial staining for locating the sampling areas during frozen section procedures of hysterectomy specimens [4]. They suggested that chromo-hysteroscopy with toluidine blue could help in pre-operative evaluation for endometrial hyperplasia & endometrial carcinoma during hysteroscopy.

Toluidine blue is an acidophilic, vital dye of the thiazine group that stains cellular nuclei containing DNA and RNA. These characteristics make toluidine blue useful for identifying premalignant and malignant tissues that have increased DNA synthesis. Toluidine blue is also used to screen for early squamous esophageal cancers.

This study was therefore planned in women with postmenopausal & perimenopausal bleeding to evaluate role of chromo-hysteroscopy guided biopsy in targeting endometrial lesions for diagnosis of endometrial pathology using toluidine blue dye.

Objective

To evaluate the diagnostic accuracy of Chromo hysteroscopy guided biopsy for endometrial hyperplasia and endometrial carcinoma.

Material and Methods

This cross-sectional interventional study was conducted over a period of one year in the Department of Obstetrics and Gynecology at King George's Medical University. Ethical clearance was obtained from Institutional Ethical Committee. Fifty women, aged above 40 years were recruited with complaints of menorrhagia, metrorrhagia, polymenorrhagia or postmenopausal bleeding. Written informed consent was taken. After general, systemic and gynaecological examination, all women were investigated for haemogram, coagulation profile and thyroid profile. Pap smear and trans-vaginal sonography (TVS) were performed. Women with thyroid disorders, coagulation disorders, cervical or vaginal pathology were excluded from the study.

- 50 women were recruited who underwent the following procedures sequentially:

Diagnostic hysteroscopy

This was done under all aseptic precautions using cervical block for analgesia. A 4 mm diameter hysteroscope was used to visualize and record the type of endometrial lining, shape of endometrial cavity, position of bilateral ostia, & presence of any growth or ulceration in the cavity.

Instillation of toluidine blue dye

The normal saline inflow was turned off, tubing was disconnected and 5 ml of 0.5% toluidine blue dye was instilled through inlet channel of hysteroscope. Inlet channel was then closed to prevent backflow. Toluidine blue was allowed to remain in the cavity for 5 minutes. After 5 minutes, inflow of normal saline was restarted and outlet opened temporarily to wash off the dye. The percentage of endometrial surface stained was recorded as less than or more than 50% of endometrial surface area.

Hysteroscopic guided biopsy

Hysteroscopic biopsy forceps was introduced through side channel and endometrial biopsy was taken first from area stained by toluidine blue dye and later from area that didn't take the stain. Each sample of endometrial tissue obtained was put in a separate vial containing

formalin and labelled as specimen I and II. Hysteroscope was withdrawn from the endometrial cavity.

Endometrial aspiration (EA) biopsy was taken from the uterine cavity using disposable Karman cannula (5 mm) attached to 20 cc syringe. The EA tissue was labelled as specimen III.

All three endometrial biopsy specimens were sent for histopathological examination (HPE). The Histological diagnosis of worst pathology (endometrial hyperplasia or endometrial carcinoma) in any of the three specimens was recorded as the final diagnosis.

Statistical analysis

Diagnostic accuracy of chromo hysteroscopy guided biopsy from stained area & unstained area, and endometrial aspiration were calculated using SPSS Version 15.0 statistical Analysis Software.

Results

Mean age of presentation of cases included in this study was 47.56 ± 8.44 years (range 40-80 years). 38 subjects (76%) were in age group >40-50 years while only 1 case was in age group >70 years (2%). 39 patients were multiparous (78%). Only a total of 2 subjects were nulliparous (4%) and 9 (18%) patients were grand-multiparous.

37 (74%) women included in this study were premenopausal and 13 (26%) women were postmenopausal. 20 (54.05%) premenopausal women had menorrhagia, 9 (24.32%) women had polymenorrhagia & 8 (21.62%) premenopausal women had metrorrhagia.

Diagnostic hysteroscopy revealed endometrial polyps in 10, submucous fibroids in 8 and ulcerative lesion in 1 out of 50 cases. The most common abnormal hysteroscopy finding was hyperplastic endometrium, seen in 29 (78.37%) cases. Hysteroscopy confirmed all the 4 cases of endometrial polyp/ submucous fibroid suspected on TVS. In addition, 7 new cases of endometrial polyp and seven new cases of submucous fibroids were diagnosed by hysteroscopy that were missed on TVS. Hysteroscopy also detected 20 new cases of hyperplastic endometrium that were missed by TVS.

Table 1 shows demographic profile and clinical presentation of all cases

Demographic factor		No. of cases	% of cases	P value
Age range	40-50	38	76%	0.33
	50-60	7	14%	
	60-70	4	8%	
	70-80	1	2%	
Mean Age	47.56 \pm 8.44 years			
Parity	Nulliparous	2	4%	0.703
	Multiparous	48	96%	
Menopausal status	Premenopausal	37	74%	0.156
	Postmenopausal	13	26%	
Religion	Hindu	44	88%	>0.05

	Muslim	6	12%	
Clinical presentation	Menorrhagia	20	40%	>0.05
	Polymenorrhagia	9	18%	
	Metrorrhagia	8	16%	
	Postmenopausal bleeding	13	26%	

Table 1: Demographic profile and clinical presentation of all cases.

Table 2 shows histopathology results of all cases. Out of 50 cases, 12 (24%) showed endometrial hyperplasia/carcinoma as a cause of abnormal uterine bleeding. Majority of cases (48%) showed hormonal pathology on histology (endometrium out of phase).

Histopathological Reports	No. of Patients	Percentage
Endometrial Cancer	2	4%
Endometrial hyperplasia with atypia (simple/complex)	2	4%
Endometrial hyperplasia without atypia (simple/complex)	8	16%
Decidualization of endometrium	3	6%
Endometrium in Phase(normal)	11	22%
Endometrium out of Phase(Hormonal pathology)	24	48%

Table 2: Results of Endometrial Histopathology (n=50).

Chromo-hysteroscopy with toluidine blue revealed that 72.41% cases of hyperplastic endometrium, 60% cases of polyps and 75% cases

S. No.	Menstrual Abnormality	Stained	Unstained	Endometrial Aspiration
1	Postmenopausal Bleeding	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma
2	Postmenopausal Bleeding	Adenocarcinoma	Adenocarcinoma	Non-secretory Endometrium
3	Postmenopausal Bleeding	Simple cystic hyperplasia	Simple cystic hyperplasia	Simple cystic hyperplasia
4	Postmenopausal Bleeding	Simple hyperplasia	Simple hyperplasia	Proliferative endometrium
5	Postmenopausal Bleeding	Simple hyperplasia	Simple hyperplasia	Simple hyperplasia
6	Menorrhagia	Proliferative endometrium	Small stromal bits	Proliferative endometrium with focal atypia
7	Menorrhagia	Proliferative	Proliferative	EIN(Endometrial intraepithelial neoplasia)
8	Metrorrhagia	Simple hyperplasia	Simple hyperplasia	Mixed endometrium
9	Polymenorrhagia	Simple hyperplasia	Simple hyperplasia	Simple hyperplasia
10	Polymenorrhagia	Simple cystic hyperplasia	Simple cystic hyperplasia	Simple cystic hyperplasia
11	Metrorrhagia	Simple hyperplasia	Simple hyperplasia	Simple hyperplasia
12	Menorrhagia	Simple hyperplasia	Simple hyperplasia	Simple hyperplasia

Table 4: Comparison of HPE results of Stained, Unstained and EA biopsy in cases with endometrial pathology (carcinoma and hyperplasia)

of fibroids had more than 50% endometrial surface stained. In contrast, none of the cases with ulcerative lesions or adhesions took up the toluidine blue stain.

Table 3 shows correlation of endometrial surface staining with histopathological findings. 75% cases with endometrial cancer or hyperplasia showed >50% endometrial surface staining as compared to 52.8% of cases without such pathology but this difference was not statistically significant difference (p=0.171). Table 4 compares histopathology of stained biopsy, unstained biopsy and EA in 12 cases with endometrial carcinoma/hyperplasia.

Histopathology Results	No cases	Cases with >50% Staining	Percentage	P value
Endometrial Hyperplasia/Cancer	12	08	75%	0.171
Proliferative/ Secretory endometrium	38	21	52.63%	

Table 3: Correlation of endometrial staining with histopathology results.

Table 5 shows comparison of Diagnostic Accuracy of Chromo-hysteroscopic guided biopsy (CHB) and endometrial aspiration. The Sensitivity, Negative predictive value and overall diagnostic accuracy of CHB and endometrial aspiration did not show any statistically significant difference.

	Stained Tissue Biopsy	Unstained Tissue Biopsy	Endometrial Aspiration	P values
Sensitivity	83.33%	83.33%	75%	0.615
Negative Predictive Value	95%	95%	92.68%	0.665
Diagnostic Efficacy	96%	96%	94%	0.646

Table 5: comparison of Diagnostic Accuracy of Chromo hysteroscopy guided biopsy with endometrial aspiration.

Discussion

Salman MC et al., evaluated 142 women with postmenopausal bleeding and found that incidence of endometrial cancer was 12.7%. In the present study, endometrial cancer was detected in 15.3% of postmenopausal women, a similar incidence [5].

Anastasiadis et al., evaluated the prevalence of endometrial hyperplasia in 1,469 women with abnormal uterine bleeding. 294/1469 women were found to have endometrial hyperplasia, giving the prevalence of 20% [6]. Our study showed also showed an overall prevalence of 20% further divided into endometrial hyperplasia with atypia (4%) and without atypia (16%).

These findings show that there is high incidence of endometrial hyperplasia and endometrial cancer in women aged above 40 years and hence there is a definite need to evaluate them sequentially with sonography, hysteroscopy and endometrial biopsy.

Paschopoulos et al., reported sensitivity and specificity of 92% and 95% for hysteroscopy in diagnosing intra-cavitary pathology like submucosal myoma and endometrial polyp, in women with abnormal uterine bleeding [7]. Sousa et al., performed hysteroscopy on postmenopausal women with AUB and revealed sensitivity of 88.9% & specificity of 98.3% in detection of endometrial carcinoma [8]. Allameh et al., reported very high diagnostic accuracy of hysteroscopy [9]. They found 93% sensitivity & 100% specificity for detection of endometrial polyps and 100% sensitivity & 96.4% specificity for submucosal fibroid. In the present study, hysteroscopy diagnosed 7 new cases of endometrial polyp and seven new cases of submucous fibroids that were not seen on TVS. In addition, hysteroscopy showed a sensitivity of 91.67%, specificity of 50%, PPV of 36.67%, NPV of 95% and diagnostic accuracy of 60% for detecting endometrial hyperplasia or carcinoma. The ability of hysteroscopy to diagnose endometrial pathology was significantly higher ($p=0.013$) in comparison to TVS.

Kucuk et al., [2,3] and Hodo Mansoura et al., [10] showed that inflammatory cells had a higher uptake of methylene blue helping in diagnosis of unexplained endometritis. We studied chromo hysteroscopy with methylene blue dye in 60 women with AUB [11]. Our study showed that 48% cases with normal endometrium and 32% cases with hormonal disturbance showed similar histology in stained biopsy, unstained biopsy and endometrial aspiration without any discrepancy. There were 11 cases with endometrial disease and eight of

these were diagnosed by the biopsy from stained tissue. The diagnostic accuracy of stained tissue biopsy to detect endometrial pathology was significantly higher than that of unstained tissue biopsy and endometrial aspiration ($p=0.006$) in our study. Out of these 11 cases, five did not show any abnormality or suspicious lesions on diagnostic hysteroscopy. This shows that chromo hysteroscopy adds to the diagnostic accuracy of hysteroscopy in about 50% cases and that is a great advantage. Alay et al. [12] performed chromo hysteroscopy with methylene blue in 38 cases of AUB. They did not find any statistically significant difference in the comparison of biopsy samples obtained from methylene-blue stained, non-stained areas and blind biopsy ($P>0.05$).

Kucuk et al., performed chromo-hysteroscopy using methylene blue in 22 women with postmenopausal bleeding [13]. It helped them diagnose 3 more endometrial pathologies that included 2 cases of endometritis & 1 case of endometrial hyperplasia. Meatumato et al., found that dysplastic cells predominantly take up methylene blue but the results were not reproduced [14].

Yahia et al., used methylene blue for chromo hysteroscopy in 50 postmenopausal women and found a sensitivity of 93.75%, specificity 27.77%, PPV 69.76%, and NPV 71.42% [15]. Chromo hysteroscopy led to the diagnosis of three more cases of endometritis and two more cases of focal endometrial hyperplasia but none of endometrial carcinoma. They concluded that Chromo hysteroscopy improves the efficacy of hysteroscopy in targeting endometrial biopsy.

Ozturk et al., conducted a cross-sectional study in 50 patients to evaluate the usefulness of staining for locating the sampling areas during frozen section procedures in the evaluation of hysterectomy specimens [4]. They concluded that the sensitivity of toluidine blue staining in the determination of endometrial hyperplasia and endometrial carcinoma was 100%, specificity was 90%, positive predictive value was 87% & negative predictive value was 100%.

In the present study, chromo-hysteroscopy was done using toluidine blue dye. 72.41% cases of hyperplastic endometrium, 60% of polyps and 75% of sub mucus fibroids showed more than 50% staining while none of the ulcerative lesions or adhesions took up the toluidine blue stain. 75% cases of endometrial hyperplasia/carcinoma showed $\geq 50\%$ endometrial surface staining while only 52.63% of cases with normal HPE showed similar staining. These findings suggest an affinity of toluidine blue towards areas with cellular hyperplasia but none of the comparisons showed any statistically significant difference.

The histopathology examination of chromo hysteroscopy guided biopsy from stained and unstained area showed similar findings in all 12 cases with endometrial carcinoma and hyperplasia. CHB diagnosed 10 cases and missed 2 cases of endometrial carcinoma and hyperplasia. On the other hand, endometrial aspiration diagnosed 9 cases and missed 3 cases of endometrial carcinoma and hyperplasia. There was no statistically significant difference in the sensitivity, negative predictive value or overall diagnostic accuracy of Chromo hysteroscopy guided biopsy and endometrial aspiration in the present study. Our study results do not replicate the results of Ozturk et al. who conducted their study on hysterectomy specimens postoperatively. We took the endometrial biopsy preoperatively using toluidine blue for chromo hysteroscopy.

The limitation of this study is that there was no hysterectomy specimen to act as gold standard for comparing histopathology results. This is not possible because hysterectomy is not indicated for all cases of endometrial hyperplasia and hormonal pathologies. The chromo

hysteroscopy guided biopsy results from stained and unstained area were compared with those of endometrial aspiration which itself can miss a few cases. Further studies are needed using some other vital dye which has specific affinity for endometrial hyperplasia or carcinoma.

Conclusion

Toluidine blue dye does not specifically stain endometrial hyperplasia or endometrial carcinoma and hence is NOT a useful stain for Chromo-hysteroscopy guided biopsy for diagnostic evaluation of such cases.

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