"Chromohysteroscopy" for evaluation of endometrium in recurrent miscarriage

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Summary

Purpose: "Chromoendoscopy" results in 34 recurrent miscarriage (MR) patients in whom conventional hysteroscopy did not show any apparent endometrial pathology. *Method:* 5 ml of 1% methylene blue dye was introduced through the hysteroscopic inlet. *Results:* The study group was classified according to the staining characteristics. Group I included 19 patients in whom focal dark staining was observed. Group II included 15 patients in whom diffuse light blue staining was observed. There was no significant difference between the two groups in age, smoking, status, BMI, number of miscarriages and in mean gestational age of the miscarriages. Time to hysteroscopy after the last miscarriage was shorter in Group I (63.9 vs 95.3 days). Then, the study group was classified according to the histopathology result. Group I included ten cases of endometritis while Group II included 24 cases with a normal histopathology. The mean number of miscarriages was higher in Group I (3.4 vs 2.5). *Conclusion:* Chromohysteroscopy improves the efficacy of hysteroscopy in RM cases and is warranted after three miscarriages in two cycles time.

Key words: Hysteroscopy; Chromohysteroscopy; Recurrent miscarriage; Endometrium.

Introduction

Recurrent miscarriage (RM), defined as the loss of three or more pregnancies earlier than 20 weeks of gestation, is of great concern to gynecologists. RM affects between 0.5 and 3% of couples [1]. Despite numerous studies the etiology of RM remains obscure, a causal factor can not be identified in half the cases [2, 3].

Known causes of RM fall into four categories: genetic, endocrinologic, immunologic and anatomic. Anatomical abnormalities can be congenital as Müllerian anomalies or can be acquired as adhesions and fibroids. Miscarriages because of uterine anatomical abnormalities are not due to the distortion of the shape of uterine cavity or to the lack of endometrial lining in the abnormal part. There is still an endometrial layer lining the abnormal part but the decidual transformation is not adequate due to inadequate vascularization [4, 5].

The diagnosis of uterine anatomical defects can be established using ultrasonography, hysterosalpingography and hysteroscopy and/or laparoscopy. Hysteroscopy has the advantage in its ability to diagnose intrauterine defects. When there is a macroscopic abnormality, it is hardly possible to miss the diagnosis, but when there is no apparent finding, the uterus is considered as normal, although endometrial function can still be defective.

Chromoendoscopy is a widely used technique in gastrointestinal imaging [6]. Over the last decade, endoscopic systems have acquired greater potency due to high resolution images owing to CCD chip technology and narrow band imaging techniques [7]. Besides imaging enhancement, gastroenterologic endoscopists use chemical agents either to identify specific epithelia, contrast or highlight subtle mucosal irregularities, or tattoo a specific mucosal site.

Unlike the gastrointestinal mucosa the endometrium is not an "absorbtive" epithelium. The endometrium does not absorb any dye under normal circumstances. However, Marconi *et al.* reported that endometrium can be stained by methylene blue except in the periovulatory phase [8]. The reason for endometrial staining is explained with apoptosis. They noted that structural damage of the cells during apoptosis would allow passage of the methylene blue dye into the cell.

The aim of the current study was to assess the value of "chromohysteroscopy" (endometrial dying during conventional hysteroscopy) for enhancement and detection of subtle endometrial changes in RMs.

Material and Method

The current study was conducted between January 2005 and August 2007. Inclusion criteria were at least two consecutive miscarriages without a known cause. There was no age limit. Women were not included if they (or their husbands) had any chromosomal abnormality, endocrinological disease or antiphospholipid syndrome. Also, women with a known uterine abnormality were excluded. Applying these criteria 37 women were included in the study. As this was a preliminary study we did not calculate a sample size prior to the study. Institutional review board approval and written informed consents were obtained.

All hysteroscopic operations were performed in the early follicular phase. In three cases hysteroscopy revealed a uterine structural abnormality which was missed in ultrasonography and/or hysteroscopy (1 polyp, 2 adhesions), and they were excluded from the study. Conventional hysteroscopy did not show any apparent endometrial pathology in the remaining 34 patients. These were included in the analysis.

When no apparent abnormality was seen in the endometrial cavity, distending medium flow was stopped and 5 ml of 1%

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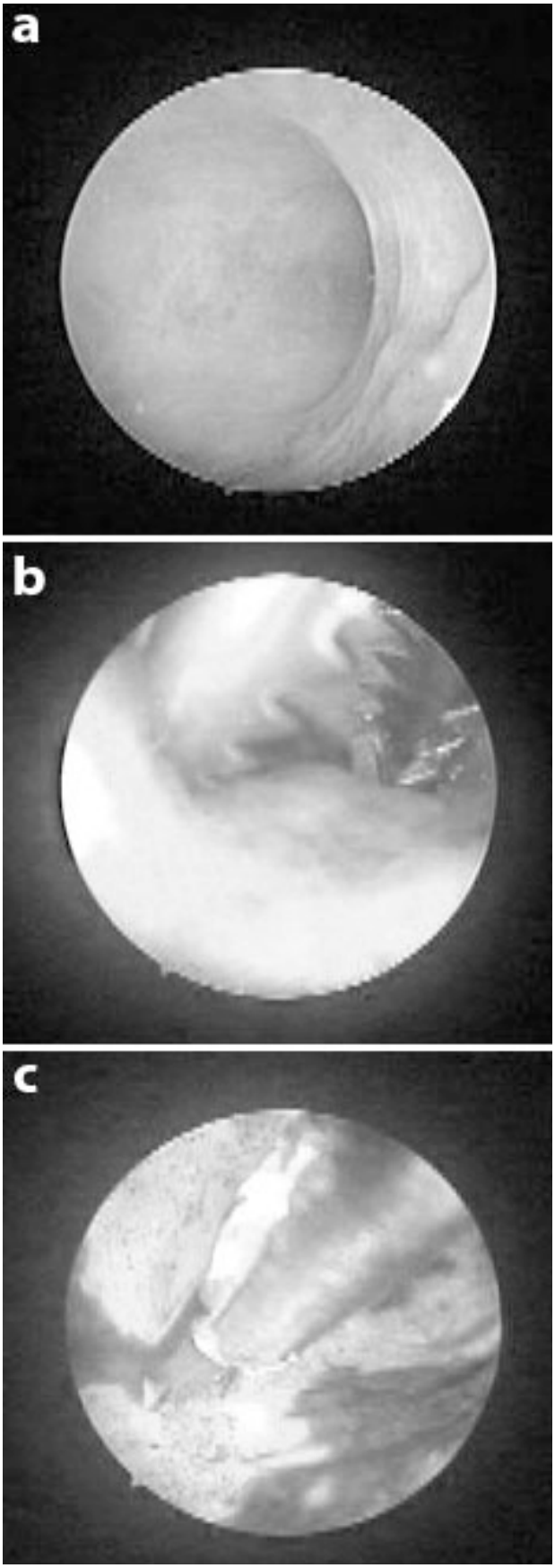

Figure 1. — a) Conventional hysteroscopic view; b) Light staining area; c) Dark staining area.

methylene blue dye was introduced through the hysteroscopic inlet. After 5 min distending medium flow was started again to wash the endometrium. The uterine cavity was visualized for any staining pattern. Diffuse light blue staining was considered normal. Focal, dark blue staining above the internal cervical ostium, regardless of size and number of stained areas, was considered a positive finding (Figure 1). Biopsies were obtained from dark stained and light stained areas and sent to pathologic examination in separate bottles.

All procedures were performed in an operating room. The classic dorsal litotomy position was employed for all hysteroscopic procedures and a 2.9 mm, 30° rigid telescope with an operative sheath of 3.5 mm was used for examination (Karl Storz, Germany). Neither speculum nor tenaculum was used in the "no-touch technique" as described by Bettochi *et al.* [9]. All patients were given intraoperative antibiotic prophylaxis with 1 g ceftriaxone (Rocephin, Roche, Istanbul).

Endometrial biopsies were obtained using hysteroscopic grasping forceps. With the jaws open, the forceps were pushed into the endometrium until sufficient tissue was grasped.

Time to hysteroscopy after the last miscarriage was between 33 and 205 days (mean 77 ± 40 days).

Results

The conventional hysteroscopy and chromohysteroscopy procedure were successful in all 34 patients. Prior to dying of the endometrium there was no apparent abnormality in any of the patients. With endometrial dying, focal dark staining areas were observed in 19 of 34 patients. Two biopsies were taken, one from the light stained area and another from the dark stained area and sent for pathological examination in separate containers. When no dark stained area was seen a single biopsy was obtained from the posterior fundal endometrium. The pathologist was blinded during the initial histopathologic examination. After completion of the study, specimens were rechecked by the same pathologist and the histopathologic diagnoses were confirmed.

The study group was classified according to the staining characteristics. Group I included 19 patients in whom focal dark staining was observed and Group II comprised 15 patients in whom diffuse light blue staining was observed. There was no significant difference between the two groups in age, smoking status, BMI, number of miscarriages and in mean gestational age of the miscarriages. There was a statistically significant difference in time to hysteroscopy ($p = 0.030$). Time to hysteroscopy after the last miscarriage was shorter in Group I (63.9 vs 95.3 days) (Table 1).

Table 1. — *Classification according to staining.*

Characteristics	Group I n = 19	Group II n = 15	p
Age (years)	27.4	27.2	NS
Smoking (%)	26.3	40	NS
BMI (kg/m^2)	20.75	22.54	NS
Time to hysteroscopy (days)	63.94	95.33	0.0030
Number of miscarriages	3	2.6	NS
Mean gestational age of miscarriages	7.75	7.68	NS

Group I: dark staining; Group II: diffuse light blue staining; NS: not significant. Comparisons were made by using the Mann-Whitney U-test.

Afterwards, the study group was classified according to the histopathology result. Group I included ten cases of endometritis while Group II included 24 cases with a normal histopathology. There was no significant difference between the two groups in age, smoking status, BMI, time to hysteroscopy and in mean gestational age of the miscarriages. This time, a statistically significant difference was found in the number of miscarriages (p = 0.008). The mean number of miscarriages was higher in Group I (3.4 vs 2.5) (Table 2).

Table 2. — *Classification according to histopathology.*

Characteristics	Group I n = 10	Group II n = 24	p
Age (years)	28.3	26.9	NS
Smoking (%)	40	29	NS
BMI (kg/m^2)	21.25	21.67	NS
Time to hysteroscopy (days)	71.50	80.42	NS
Number of miscarriages	3.4	2.5	0.008
Mean gestational age of miscarriages	7.90	7.62	NS

Group I: endometritis; Group II: normal histopathology; NS: not significant. Comparisons were made by using the Mann-Whitney U-test.

Stratification of the patients according to the mean gestational age of miscarriage did not produce any significant difference (Table 3).

Table 3. — *Stratification according to gestational age of miscarriages.*

Characteristics	< 7 weeks	≥ 7 weeks	p	< 10 weeks	≥ 10 weeks	p
Dark staining (%)	58.3	54.5	0.832	56.7	50	1
Endometritis (%)	25	31.8	1	30	25	1

Comparisons were made by using Fisher's exact test. Significance was a p value < 0.05.

Discussion

Ultrasonography, sonohysterography, hysterosalpingography and 3D ultrasonography can all show uterine and corresponding endometrial anatomy. However the presence of an endometrial lining does not assure normal endometrial function. Hysteroscopic visualization is recommended for a direct and closer look at the endometrium in RM.

Although RM is often defined as three or more consecutive pregnancy losses some investigators have included women with two miscarriages in their series [10, 11]. Weiss *et al.* compared hysteroscopic findings after either two or three miscarriages [12]. The rate of uterine abnormalities was not significantly different and hysteroscopy might be justified after two miscarriages. Women with two miscarriages were also included in the current study.

The role of infection in first trimester RM is controversial. Associates of RM with high titers of IgG antibody to chlamydia have been reported [13]. Summers reported that infection is an occasional cause of sporadic spontaneous miscarriage and, consistent with statistical probability [14]. La Sala *et al.* reported an incidence of 2% endometritis among 100 women with two consecutive IVF failures [15]. The incidence of endometritis in our series was higher and was likely related to better targeting of the biopsy by endometrial dying, and/or examination of the tissues by a dedicated gynecopathologist. Zeyneloglu *et al.* reported that observation of micropolyps in hysteroscopy was a significant predictor of miscarriage after IVF-ET [16]. Although the incidence of endometritis was not reported, patients were treated by ciprofloxacin when endometritis had diagnosed. Micropolyposis is a common finding in endometritis [17].

Local damage to the endometrium, as in incomplete Asherman's syndrome, produces patchy fibrosis without a significant amount of intrauterine adhesions [1]. Endometrial responsiveness to steroid hormones is reduced in affected areas. Those areas contain defective endometrial cells which allow methylene blue into the cell. Removal of those areas leads to replacement by healthy cells and responsiveness is restored, and eventually successful implantation is achieved. This theory is supported by the study of Barash *et al.* [18] who showed that local injury to the endometrium significantly increased the pregnancy rate in IVF. It can be speculated that local injury induced by a biopsy catheter might have removed the defective endometrium to be replaced by a new cell line.

Japanese medicine is expertised in chromoendoscopy for the early detection of gastrointestinal premalign/malign diseaes. The current study was inspired from their approach to enhance subtle mucosal changes. Conventional hysteroscopy has its limitations in evaluating endometrial cell integrity. Chromohysteroscopy increases the effectiveness of the intervention. Structural endometrial cell damage as indicated by dark staining was seen in the majority of recurrent early miscarriage cases. This might prevent either adhesion or invasion of a blastocyst into the decidua properly. The majority of dark stained areas were diagnosed as endometritis in pathological examination. It has been shown that local endometrial defects can cause miscarriage. It has also been shown that septal endometrium or endometrium covering a submucous fibroid responds suboptimally to steroid hormones and shows defective development [5, 19].

In conclusion, chromohysteroscopy improves the efficacy of hysteroscopy in RM cases. The results of this study indicate that chromohysteroscopy is warranted after three miscarriages in two cycles time. Gestational age of miscarriages does not affect the results. Larger studies are needed to draw stronger conclusions and probable routine use of endometrial dying.

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