Is There a Role of Chromoendoscopy in the Early Detection of Precancerous Lesions of the Esophagus?

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Abstract: Background: Barrett's esophagus (BE) is a condition that is premalignant for adenocarcinoma of the esophagus. Early detection of Barrett's metaplasia and dysplasia is very important to decrease the mortality and morbidity from esophageal cancer. Chromoendoscopy using methylene blue (MB) has been used in BE evaluation. **Objective:** To evaluate the role of chromoendoscopy in the early detection of precancerous lesions of oesophagus. **Methods***:* This study was conducted on 32 patients who give long history of GERD or history of Barrett's oesophagus. The patients were divided into two groups; *Group I:* 10 patients were selected for conventional and then chromoendoscopy, after which biopsies were taken from stained and unstained areas. 6 patients were selected for chromoendoscopy only, so biopsies were taken from stained areas of the esophagus. *Group II:* 16 patients were selected for conventional endoscopy and biopsies were taken by 4 quadrant technique. Conventional and chromoendoscopic assessments were compared with histopathologic examinations. **Results:** There was no significant statistical difference as regards age, gender & duration of symptoms between both groups. The sensitivity of chromoendoscopy for Barrett's epithelium was superior to that of conventional endoscopy. Stained biopsies were superior to unstained biopsies in terms of sensitivity for Barrett's epithelium and esophageal carcinoma. **Conclusion:** The data presented suggest chromoendoscopic examination may provide a higher sensitivity for the diagnosis of BE and can indicate the correct location for taking biopsies.

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1. Introduction:

Gastro-esophageal reflux disease (GERD), is defined as chronic symptoms or mucosal damage produced by the abnormal reflux in the esophagus [(1)]. Barrett's esophagus (BE) refers to an abnormal change (metaplasia) in the cells of the lower end of the esophagus thought to be caused by damage from reflux esophagitis. The normal lining of the esophagus (squamous epithelium) is replaced by an intestinal-type lining (columnar epithelium) [(2)]. BE is considered a premalignant condition because it is associated with an increased risk of esophageal cancer (more specifically, adenocarcinoma). The metaplastic columnar cells may be of two types; gastric or colonic. A biopsy of the affected area will often contain a mixture of both. Colonic-type metaplasia is the type of metaplasia associated with risk of malignancy in genetically susceptible people [(3, 4)]. Based on the length of the columnar segment at endoscopy, BE has been separated into two broad categories: long-segment and short-segment. The current surveillance guidelines remain the same for both short- and long-segment Barrett's esophagus [(5)].

The most appropriate method for both diagnosis and surveillance of BE is endoscopy. Its sensitivity is higher than other comparative techniques, such as barium based studies, Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI). Endoscopic screening programs can be beneficial in both highlighting patients with BE from those with chronic GERD, as well as monitoring patients with established disease who are at risk of progressing to adenocarcinoma of the esophagus [(6)].

Los Angeles (LA) classification was used for the endoscopic diagnosis of GERD. According to this classification, GERD is divided into four grades, designated A-D [(7)].

Although screening for BE relies largely on established endoscopic techniques, it remains an area of controversy for several reasons including low prevalence and the invasiveness of endoscopy, as well as a lack of an easily identifiable demographic group [(8)].

Chromoendoscopy refers to the topical application of stains or dyes at the time of endoscopy in an effort to enhance tissue characterization, differentiation, or diagnosis. The stains that are used for chromoendoscopy are classified as *absorptive* (Lugol's solutions and methylene blue), *vital* (as indigo carmine), and *reactive stains* (as congo red) [(9)].

Methylene blue (MB) stains the normal absorptive epithelium of the small intestine and colon. The absence of staining in these tissues usually indicates the presence of metaplastic, neoplastic, or inflammatory change. MB also stains absorptive intestinal type metaplasia of the esophagus and

stomach. It has been used primarily in BE [10] and, to a lesser extent, for the detection of gastric intestinal metaplasia [11] and dysplasia in chronic ulcerative colitis [12].

Positive staining for Barrett's intestinal metaplasia is defined as the presence of dark blue–stained mucosa that persists despite vigorous irrigation [13], whereas staining pattern heterogeneity and decreased stain intensity suggest Barrett's high-grade dysplasia or cancer [14]. The use of MB staining in conjunction with magnification or high-resolution endoscopy may improve the diagnostic yield, whereas inadequate staining technique and inflammation may contribute to errors in interpretation [15].

Aim of the study:

The study was performed to evaluate the role of chromoendoscopy in the early detection of precancerous lesions of oesophagus.

2. Patients & Methods:

This study was conducted on 32 patients who gave long history of GERD or history of BE. They were recruited from the Internal Medicine Department at Tanta University Hospitals. A written informed consent was taken from all patients and the study was approved by local ethical committee at Tanta Faculty of Medicine.

The patients were divided into two groups: ***Group I:*** 10 patients were selected for conventional and then chromoendoscopy, after which biopsies were taken from stained and unstained areas. 6 patients were selected for chromoendoscopy only, so biopsies were taken from stained areas of the esophagus. ***Group II:*** 16 patients were selected for conventional endoscopy and biopsies were taken by 4 quadrant technique.

All patients were subjected to thorough history taking as regards age, sex, duration of symptoms, previous ablative therapy on esophagus, or any associated diseases, ***complete clinical examination and investigations including*** full blood count, complete liver functions, blood urea, serum creatinine, INR, ECG, pregnancy test for married female in child bearing period, and pelviabdominal ultrasonography. **A clean container labeled with the patient name was used for collecting biopsies.**

Method of taking biopsies: ***Principle:*** MB is a blue dye that is readily taken up by intestinal-type absorptive cells in the GIT. Chromoendoscopy of the distal esophagus with 1% MB was performed on 16 patients. In 10 patients of them, biopsies were taken from stained and unstained areas and the other 6 patients biopsies were targeted toward only stained or macroscopically abnormal mucosal areas. In other 16 patients, unstained columnar epithelium lined esophagus was sampled by obtaining 4-quadrant biopsy specimens at 2 cm intervals. ***Procedure:*** **All the patients were sedated during endoscopic examination. Removal of surface mucus** with an agent such as a 10% solution of N-acetylcysteine by spraying it on the Barrett's mucosa with a special washing catheter that creates a fine mist. Next, a **0.5% solution of MB is sprayed on the columnar lined epithelium** (CLE) before vigorous washing with tap water. A 1- to 2-minute wait was needed to allow the mucolytic agent to work and also for the dye to be absorbed. The volumes of mucolytic agent and methylene blue dye required vary according to the length of the columnar mucosa being stained. The original technique involves the use of approximately 10 mL of acetylcysteine and 20 mL of methylene blue dye for every 5 cm of circumferential CLE. **The endpoint of staining** is the point at which the surrounding or adjacent squamous epithelium is free of dye and the staining pattern within the CLE appears stable. **Positive staining** is defined as the presence of blue-stained, noneroded mucosa that persists despite vigorous water irrigation. MB staining adds an average of 5 to 7 minutes to the procedure time. **All of the biopsies were fixed immediately with 80% alcohol. Biopsies are embedded with paraffin. Serial sections were made and stained with H&E for histopathological analysis.** These slides were coded and evaluated by the pathologist, and the code was broken after all of the histopathological analyses were completed. **Histopathological diagnoses and evaluations were made according to** the cellular morphological changes and tissue architecture. The epithelium was graded as normal, esophagitis, Barrett's esophagus (intestinal metaplasia), dysplasia and carcinoma (adenocarcinoma or squamous cell carcinoma). Conventional endoscopic or chromoendoscopic diagnoses were compared with histopathologic diagnosis.

Statistical analyses:

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS V.16. Probability values of less than 0.05 were considered of statistical significance [16].

3. Results:

The demographic data of the 16 patients of group I showed that 12 were males and 4 were females with mean (± SD) age of 48.06 ± 14.56 years. The demographic data of the 16 patients of group II showed that 11 were males and 5 were females with mean (± SD) age of 49.43 ± 16.74 years. Comparison between all studied groups as regard age and sex were statistically insignificant (Tables 1 & 2).

As regards duration of symptoms; in group I, the mean duration of symptoms of GERD (± SD) was 15.31±3.84 months. In group II, the mean duration of

symptoms of GERD (±SD) was 17.12±7.10 months. There was no statistically significant difference between both groups as regards duration of symptoms (*p*.value 0.824). *As regards symptoms;* heartburn was found in 24 patients, regurgiatation in 25 patients, dysphagia in 2 patients, chest pain in 13 patients, nausea in 7 patients, vomiting in 4 patients and only 1 patient presented with upper GI bleeding. Comparison between all groups as regards symptoms was statistically insignificant (*p.* value 0.274) (Tables 3 & 4).

As regards the endoscopic findings; 13 patients had incompetent cardia, 12 patients had sliding hiatal hernia, 26 patients had different grades of reflux esophagitis from grade A to D according to LA classification, 4 patients of them were presented by lower esophageal ulcerations and one patient was presented by a hardly passable stricture. Comparison between studied groups regarding endoscopic findings was statistically insignificant (Table 5).

As regard to types of BE found in the study during endoscopic examination; we found that 20 patients had short segment BE and 7 patients had long segment BE while the remaining 5 patients had only signs of reflux esophagitis**.** The difference between both groups was statistically insignificant (*p*.value 0.343) (Table 6).

As regards histopathological data; six diagnostic categories were defined: normal, esophagitis, esophagitis with metaplastic columnar cells (without goblet cells), BE, low-grade dysplasia and high-grade dysplasia. No cases of esophageal adenocarcinoma were found during this study. MB targeted biopsies showed a higher detection for Barrett's epithelium in group (I) in 10 patients who underwent both conventional and chromoendoscopy. 3 cases of low-grade dysplasia and one case of high-grade dysplasia were found, while one case of low-grade dysplasia appeared in 4 quadrant biopsies taken during conventional endoscopy (Table 7). In the comparison between groups (I) and group (II), we found 4 cases of esophagitis with normal stratified squamous epithelium and one case of metaplastic columnar epithelium (but without goblet cells) from the 16 patients of group (I). While in group (II), 3 normal cases and no cases of metaplastic columnar epithelium were found among the 16 patients of the studied group (Table 8) and there was statistically significant difference between the studied groups.

In group (I), MB targeted biopsies showed a higher sensitivity (100%) than conventional biopsies (85.7%). The same data were found when groups (I) and (II) were compared, where the sensitivity of MB in group (I) was (81.8%) while specificity was (4o%). However, detected cases of dysplasia and metaplasia were equal in both groups (Table 9).

Table (1): Comparison between the studied groups regarding age.

Age.	**GI**	**GII**
Mean	48.06	49.43
(±)SD	14.56	16.74
t. test	**0.248**	
***p*. value**	**0.806**	

P value > 0.05 (non significant)

Table (2): Comparison between studied groups regarding sex.

			Sex		
			Male	**Female**	**Total**
GI		N	12	4	16
		%	75	25	100
GII		N	11	5	16
		%	68.8	31.3	100
Total		N	23	9	32
		%	71.9	28.1	100
Chi-Square	**X^2**	**0.155**			
	***P*-value**	**0.635**			

Table (3): Comparison between studied groups regarding duration of symptoms (months).

Duration (Months)	**GI**	**GII**
Mean	15.31	17.12
(±)SD	3.84	7.10
t. test	**0.224**	
***p*. value**	**0.824**	

Table (4): Comparison between studied groups regarding symptoms

Symptom		G I (n=16)		GII (n=16)	
		N	%	N	%
Heartburn		13	81.25	11	68.75
Regurgitation		13	81.25	12	75
Dysphagia		1	6.25	1	6.25
Chest pain		6	37.5	7	43.75
Nausea		3	18.75	4	25
Vomiting		2	12.5	2	12.5
Upper GI bleeding		1	6.25	0	0
Iron deficiency anemia		0	0	0	0
Chi-Square	**X2**	**1.638**			
	P-value	**0.274**			

Table (5): Comparison between studied groups regarding endoscopic findings.

Endoscopic findings		G I		GII	
		N	%	N	%
Incompetent cardia		8	50	5	31.25
Sliding hiatal hernia		8	50	4	25
Reflux esophagitis					
Grade A		1	6.25	2	12.5
Grade B		7	43.75	6	38.5
Grade C		5	31.25	4	25
Grade D		1	6.25	0	0
Lower esophageal ulceration		0	0	4	25
Hardly passable stricture		0	0	1	6.25
Chi-Square	**X2**	**1.520**			
	***P*-value**	**0.362**			

Table (6): Comparison between studied groups regarding type of Barrett's esophagus during endoscopy

		Type of Barrett's			
		Short segment	**Long segment**	**No Barrett's**	**Total**
GI	N	9	3	4	16
	%	56.2	18.8	25	100
GII	N	11	4	1	16
	%	68.7	25	6.3	100
Total	N	20	7	5	32
	%	62.5	21.9	15.6	100
Chi-Square	**X^2**	**2.143**			
	***P*-value**	**0.343**			

Table (7): Comparison between histopathological findings of conventional and chromoendoscopy in the same group (I).

			Four quadrant biopsy	**MB targeted biopsy**	**Total**
Normal stratified squamous epithelium		N	2	0	2
		%	100	0	100
Esophagitis with normal st. squamous epithelium		N	4	3	7
		%	57.1	42.9	100
Esophagitis with metaplastic colum. epith. (without goblet cells)		N	1	1	2
		%	50	50	100
Metaplasia		N	2	2	4
		%	50	50	100
Barrett's with low grade dysplasia		N	1	3	4
		%	24	75	100
Barrett's with high grade dysplasia		N	0	1	1
		%	0	100	100
Total		N	10	10	20
		%	50	50	100
Chi-Square	**X^2**	**4.143**			
	***P*-value**	**0.015**			

Table (8): Comparison between methylene blue targeted biopsies in group (I) and 4 quadrant biopsies in group (II)

			GI	GII	Total
Normal stratified squamous epithelium		N	0	3	3
		%	0	100	100
Esophagitis with normal st. sq. epith.		N	4	2	6
		%	66.7	33.3	100
Esophagitis with metaplastic columnar epithelium (without goblet cells)		N	1	0	10
		%	100	0	100
Barrett's metaplasia		N	4	4	8
		%	50	50	100
Barrett's with low grade dysplasia		N	5	5	10
		%	50	50	100
Barrett's with high grade dysplasia		N	2	2	4
		%	50	50	100
Total		N	16	16	32
		%	50	50	100
Chi-Square	**X^2**		**5.214**		
	***P*-value**		**0.041**		

Table (9): Comparison between sensitivity of conventional and chromoendoscopy in the same group (I).

	Conventional biopsy	**MB targeted biopsy**
Sensitivity	85.7	100
50	100	33.3
Positive predictive value	75	50
Negative predictive value		

4. Discussion:

In BE, the stratified squamous epithelium that normally lines the distal oesophagus is replaced by an abnormal columnar epithelium that has intestinal features. It is found in 6% to 18% of patients undergoing upper GI endoscopy for symptoms of reflux disease. The abnormal epithelium (called specialized intestinal metaplasia) usually shows evidence of DNA damage that predisposes to malignancy [(17)]. After the first destruction of squamous mucosa by gastric acid or bile,the second re-epithelization of the lower esophagus may be fulfilled by pieuripotent basal cells,which may be the progenitors of Barrett's epithelium [(18)].

The groups at high risk for BE mainly consist of patients with GERD especially those with long duration and increased frequency of symptoms due to esophageal dysmotility, patients above 50 years, obesity, alcohol use, smokers, and patients with large hiatal hernia [(19)]. These are well known precursors of esophageal adenocarcinoma with a risk of 18% for low- grade dysplasia and 34% for high grade dysplasia [(20)].

In our study, a total of 32 patients were enrolled. The patients were divided into two groups: Group I: 10 patients were selected for conventional and then chromoendoscopy, after which biopsies were taken from stained and unstained areas. 6 patients were selected for chromoendoscopy only, so biopsies were taken from stained areas of the esophagus. Group II: 16 patients were selected for conventional endoscopy and biopsies were taken by 4 quadrant technique.We found that the sensitivity of chromoendoscopy for Barret's epithelium was superior to that of conventional endoscopy, with no statistically significant difference in specificity in both methods.These findings are in agreement with ***(Ormeci, et al. 2007)*** who performed a study on 109 patients and found increased sensitivity of chromoendoscopy for Barret's epithelium. However, there was no statistically significant difference between both methods in the diagnosis of esophagitis or esophageal carcinoma [(21)].

Kiesslich et al. (2003), Ragunath et al. (2003),and ***kouklakis(2003)*** reported high sensitivity (91%-98%) and variable specificity (43%-97%) [(12,13,22)], whereas smaller studies ***[Breyer et al. (2000), Dave et al. (2001)]*** reported unsatisfactory results (sensitivity of 53%-72% and specificity of 32%-51%) [(23, 24)].

Differences in the study design, the technique of MB staining, the interpretation of staining

patterns, and the endoscopist's experience with vital staining have contributed to the inconsistencies in the results. To improve the technique, endoscopists have used high-magnification endoscopy, together with MB staining to improve the characterization of the esophageal mucosal pit pattern and to increase the specificity for detection of BE to 92% to 100% [25].

Even more controversial is the role of MB chromoendoscopy for improving the diagnosis of dysplasia in BE. Two studies ***[Canto et al. (2000), Gossner et al. (2000)]*** [10,20] showed MB-directed biopsy to be significantly better than random biopsy for the diagnosis of dysplasia, but two other studies ***[Ragunath et al. (2003) & Wo et al. (2001)]*** [13, 26] did not confirm these results.

In the comparison of group I and group II, we found 4 cases of esophagitis with normal stratified squamous epithelium 1 case of metaplastic columnar epithelium (but without goblet cells). While in group II, 3 normal cases, and no cases of metaplastic columnar epithelium were found among the 16 patients of the studied groups.

Lim et al. **(2006)** reported the results of another study that compared MB chromoendoscopy with random biopsy for the detection of dysplasia in BE. The investigators randomly assigned patients with a history of Barrett's dysplasia to either MB-directed biopsy or random biopsy before repeating the alternative technique within 6 months. Of the 30 patients who completed the study, a random biopsy found dysplasia in 17 patients, whereas MB-directed biopsy of unstained areas found dysplasia in only 9, regardless of what technique was used first. The investigators concluded that MB chromoendoscopy is "less sensitive in detecting dysplasia," and they discouraged its use during routine surveillance of BE. However, this study had many limitations including the long interval between the 1st and 2nd biopsy procedures [27].

Like other techniques in endoscopy, interpretation of the results of chromoendoscopy is operator dependent, regardless of what vital stain is used. The technique of chromoendoscopy is simple, but interpretation of staining results remains a chanllenge.

There are few medical centers in the world that routinely perform MB chromoendoscopy and teach their trainees. Hence, "on-the-job" training appears to be the most practical approach to learning chromoendoscopy outside centers with special interest. With the lack of formal training in chromoendoscopy, the outcomes of vital staining for the diagnosis of GI neoplasia will predictably be varied and operator dependent.

Conclusion:

From this study, we concluded that chromoendoscopic examination may provide a higher sensitivity for the diagnosis of BE and can indicate the correct location for taking biopsies where dysplasia or early esophageal cancer is suspected. Therefore, chromoendoscopy provides a higher diagnostic sensitivity with fewer biopsies.

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