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Review Article

Clinical Review on Dyes Used in Chromoendoscopy

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1. Abstract

Chromoendoscopic technique dates to 1970, when various dyes were used on the tissue lining the GI tract to detect subtle lesions that are hard to detect using normal endoscopic imaging. Applied colour enhances tissue characterisation thus providing easier identification of pathological condition. Chromoendoscopic systems improve the identification of minute changes in the surface pattern by improving the contrast of raised and deepened areas. Based on the type of stain used different types of epithelia can be easily differentiated. This is particularly helpful in surveillance programmes aiming to detect dysplasia and pre-neoplastic lesions [e.g. in Barrett's Oesophagus (BO) or Inflammatory Bowel Disease (IBD)] with the diagnostic yield of targeted 'smarter' biopsies being superior to random biopsies, thus reducing the histopathologic workload and potentially offsetting the costs for additional procedure time. This review discusses in detail various stains equipment and drawbacks of chromoendoscopy.

2. Keywords: Chromoendoscopy; Dye; Spray catheters

3. Introduction

Gastro intestinal tract is a hollow muscular tract starting from mouth and continuing with oesophagus to stomach, small intestine, colon rectum and ending with anus along with supporting organs like liver, gall bladder and pancreas. The intraluminal pH varies all along the GI tract and is rapidly changing. Starting with neutral pH 7 at mouth and oesophagus; it turns to highly acid pH 1-2.5 in the stomach; to about pH 6 in the duodenum. This pH gradually increases to pH 7.4 in the terminal ileum; and pH drops to 5.7 in the caecum, but again gradually increases, reaching pH 6.7 in the rectum [1]. With about 20 feet of small intestine; approximately 30 feet in length from mouth to anus; hosting about 400 species of bacteria; food gets digested by various mechanical actions and chemical reactions for production of nutrients and energy.

Improper diet patterns (increased consumption of refined sugars, fats, fast foods, fatty acids and oils), environmental conditions (pollution, smoking), lack of proper sanitation, increased use of antibiotics etc. lead to a higher chance of developing a GI disease. Common symptoms of Gastro Intestinal (GI) disorder include abdominal pain, nausea, diarrhoea, constipation, vomiting, bloating, passage of blood or food through faeces. Structural or functional abnormality in GI tract is mostly represented by one or more of the above symptoms. Major causes for GI disorders identified are inflammation, infection, malignancy or structural disorders [2].

4. Diagnostic Tests

For the diagnosis of human gastro intestinal tract diseases different diagnostic tests like clinical examination, blood tests, stool analysis, abdominal ultra sound, CT scan and MRI etc. are available.

4.1. Lab Tests

- Stool culture test: Presence of abnormal bacteria in the digestive tract that may cause diarrhea and other problems. These can be identified by a stool culture test.
- Fecal occult blood test: Hidden (occult) blood in the stool cab is determined by this test.

4.2. Imaging Tests

- Ultrasound: Ultrasound is a diagnostic imaging technique that uses high-frequency sound waves and a computer to create images of blood vessels, tissues, and organs.
- Magnetic Resonance Imaging (MRI): MRI is a diagnostic test that uses a combination of large magnets, radiofrequencies, and a computer to produce detailed images of organs and structures within the body.
- Endoscopy: Procedure in which inside of the body is examined using an instrument called an endoscope.

5. Endoscopy

Endoscopy is now a primary component of medical diagnostic process is an everyday tool in current medical environments. In Greek, the term "endo" means "within inside" and its usage date to 1860 for looking deep inside the body. Endoscopy is defined as "A medical device used for visual examination of the interior of a body cavity or a hollow organ such as the colon, bladder, or stomach. It is a rigid or flexible tube fitted with lenses, a fibre optic light source, and often a probe, forceps, suction device, or other apparatus for examination or retrieval of tissue [3].

Based on the region being examined, endoscopy is being termed as Gastro-Intestinal endoscopy (GI endoscopy) for various digestive organs. For observing upper and lower abdomen comprising oesophagus, stomach and duodenum, it is termed as esophago-gastro-duodeno-scopy, colonoscopy or sigmidoscopy for large intestine or colon, rectoscopy for bile duct and rectum and anoscopy for anus. For observing respiratory tract, it is termed as rhinoscopy for nose and bronchoscopy for lower respiratory tract. Its termed as octoscopy for visualising the ear and cytoscopy for urinary tract and gynoscopy for various reproductive organs.

5.1. History of Endoscopy

Desire to look deep into human body cavity, and study started centuries ago [4]. To obtain information on human body in 1585, Giulio Cesare Aranzi an Italian physician used sunlight to observe the body cavity. Several such examinations continued even in 1800 by Waldenburg and Kussmaul. Later, Philipp Bozzini an Italian physician used candle light in his device 'Lichtleiter'. He was followed by Anton in Jean Desormeaux who used chemical reactions along with candle, like mixture of alcohol and turpentine, which further increased the intensity of light. Desormeaux further used condenser and he is regarded as father of endoscopy for his first successful operative endoscopy in living subjects. John Fisher from Boston, described a similar instrument, using candlelight, a tubular speculum, and a system of mirrors in 1827.

Later, Julius Bruck (1840-1902), a dentist, inserted light source into human body for the first time followed by Max Nitze who used miniaturized Edison's filament globe and created the first cystoscope in 1877. This was followed by Kelling in 1898 who for the first time recorded flexible esophago scope. Later, in 1908 hysteroscopy was used by Charles David.

After many patent wars, in the year 1952 Fourestier used a rigid quartz rod of 1.5mm in diameter which was inserted into a 2 mm stainless steel tube. Advancements in physics, optics, computers and electronics have increased the flexibility, image quality, and operational reach of endoscopes. In 1954, Basil Hirschowitz and Larry Curtiss developed fibre optic endoscope Later in 1959 Hopkins developed rod lens system.

By the 1960's flexible sigmoidoscopies were being developed in the USA and Japan and in the late 1960's and early 1970's the first cases of total fiberoptic colonoscopy were being reported. Current day endoscopy advanced with a built-in video camera using Charge Coupled Device (CCD). Internal images are converted to electronic signals and displayed on a digital monitor increasing diagnostic accuracy and enabling easier view for several doctors at a time. Through various endoscopic advances available today all the data can be recorded and retrieved when required.

5.2. Indications for Endoscopy

GI endoscopy is generally performed to visually examine an organ without an incision and determine the cause of any underlying reason of an abnormal symptom. Diagnostic observations are generally made to determine existence of any focal benign or malignant lesions, diffuse mucosal changes, luminal obstruction, motility, and extrinsic compression by contiguous structures.

5.2.1. Diagnostic Indications for Upper GI Endoscopy (EGD): EGD offers an excellent view of mucosal surfaces of the esophagus, stomach, and proximal duodenum [5]. This technique is generally used for the diagnosis of the conditions like

- Isolated dysphagia and/or odynophagia
- Persistent isolated nausea or vomiting

- Dyspepsia
- Chronic anaemia and/or iron deficiency
- Acute gastrointestinal bleeding originating in the upper GIT
- Gastro-esophageal reflux
- Barrett's esophagus
- Peptic ulcer
- Duodenal biopsy

Sequential or periodic EGD may be indicated for surveillance of malignancy in patients with premalignant conditions (e.g., BE, polyposis syndromes, gastric adenomas, or previous caustic ingestion).

5.2.2. Indications for Lower GI Endoscopy: Lower GI endoscopy, also termed as enteroscopy is performed when colonoscopy and EGD cannot provide proper information [6]. This allows the visualization of a greater extent of the small intestine.

- Evaluation of the source of GI bleeding not identified by EGD or colonoscopy.
- Evaluation of an abnormal radiographic imaging study of the small intestine.
- Localization of known or suspected small intestine lesions.
- Therapy of small intestine lesions beyond the reach of a standard endoscope.
- Tissue sampling from the small intestine.

5.2.3. Indications for colonoscopy: Visualization of large intestine for possible causes of things like abdominal pain, rectal bleeding [7], or changes in bowel habits is colonoscopy and is most commonly used diagnostic aid to prevent colorectal cancer.

- Unexplained GI bleeding
- Unexplained iron deficiency anemia
- Screening and surveillance for colonic neoplasia
- For dysplasia and cancer surveillance in select patients with long-standing ulcerative or Crohn's colitis
- Clinically significant diarrhea of unexplained origin
- Foreign body removal
- Excision or ablation of lesions

5.2.4. Indications for GI Endoscopy in Paediatric Population: Size of gut is relatively small in paediatrics as compared to adult gut both in length and diameter [8]. Fully functional endoscopes with external diameter of 4.9 mm are being used in children from birth. Children with suspected coeliac disease, reflux esophagitis and suspected inflammatory bowel disease require diagnostic endoscopic procedures. Introduction of routine endoscopic surveillance programmes can help to decrease the incidence and reduce the mortality due to GI disorders.

5.3. Therapeutic Applications of Endoscopy

With advancement in technology, endoscopy is now not only being used as a diagnostic device but is also equally popular as a therapeutic device [9, 10]. Endoscopy in therapeutic field is the least invasive technique which provides equivalent outcomes without major surgery, including scars, postoperative pain, and any other continuous reminder of surgery. Applications of therapeutic endoscopy in gastroenterology include:

- Gastrointestinal bleeding: Hemostasis includes photocoagulation, electro coagulation, thermo coagulation and injection method.
- Colonic polyp: Colonoscopic polypectomy is a widely used technique in which polyps are being removed and thus decreasing the incidence of colonic cancer. The "hot biopsy" forceps can be used to excise polyps of up to 6 mm. larger polyps can be removed safely by snare electrocautery and retrieved for histologic study.
- Choledocholithiasis: Presence of gallstones in the common bile duct is choledocholithiasis and these are removed by biliary endoscopic sphincterotomy technique.

6. Chromoendoscopy

Chromoendoscopy (CE) is a diagnostic procedure in which colour is applied in the form of staining agent on to gastrointestinal tract to enhance the imaging process. This technique dates to 1970, when dyes or stains were used on the tissue lining the GI tract to detect subtle lesions that are difficult to detect using normal endoscopic imaging. Applied colour improves tissue characterisation and thus provides easier identification of pathological conditions in esophagus, stomach and large intestine. This technique is less evaluated for small intestinal region as insertion of the endoscope into the distal small intestine is challenging due to its multiple folds. Hence, small intestine is evaluated using capsule endoscopy, double balloon endoscopy etc.

Minute changes in the surface pattern can also be noted by enhancing the contrast of raised and deepened areas by using CE [11]. Stain-

ing substances required for chromoendoscopic spraying are easily available at a low cost. However, routine spray solution preparation is cumbersome and messy. Dye spilling and improper staining are common problems encountered in this method. With slight modifications in procedures this technique can provide easier tissue characterisation.

Based on the type of stain used, different types of epithelia can be easily differentiated. CE is particularly helpful in surveillance programmes aiming to detect dysplasia and pre-neoplastic lesions in BE [12] or IBD [13].

Common applications of chromoendoscopy are:

- Identification of squamous cell carcinomas of the esophagus.
- Screening of Barret's esophagus and dysplasia.
- Identification of early gastric cancers.
- Characterization of colonic polyps and colorectal cancer.
- Screening for dysplasia in individuals with ulcerative colitis.

6.1. Dyes

- Staining substances used in CE are classified into three different types based on the mechanism of stain as:
- Absorptive or vital dyes
- Non-absorptive or contrast dyes
- Reactive dyes
- **Absorptive Dyes:** These agents have an affinity for certain mucosal elements and are absorbed into the tissues.

6.1.1. Acetic Acid: Acetic acid, a weak acid is a non-coloring agent. When it is sprayed onto the tissue, it augments the structural surface pattern like a contrast agent. Disulphide bridges of glycoproteins among the mucus layers are easily damaged by acetic acid, and this result in a reversible denaturation of proteins. It has been used in colposcopy for several years as it brings out dysplastic squamous lesions of the cervix [14]. As represented in (Figure 1). endoscopic patterns can be easily correlated with histologic identification of specialized intestinal metaplasia using acetic acid.

6.1.2. Lugol's Iodine: Esophageal epithelium has capacity for staining with Lugol's solution due to the glycogen content of the squamous epithelium [15]. Normal squamous esophageal epithelium, is stained brown or dark brown with Lugol's solution and shows a "silk-crape" like surface appearance while cancerous or inflammatory epithelium of the stomach and esophagus do not stain and can be used for detection of early esophageal cancers. Staining with Lugol's iodine is represented in (Figure 2).

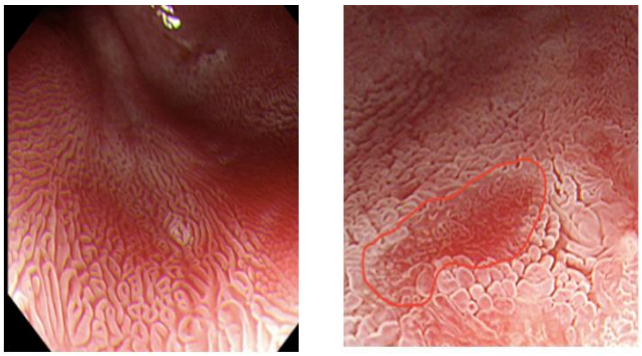

Figure 1: Acetic acid chromoendoscopy [20].
A) Uniform evenly spaced pits and normal pit density in BE
B) Compact pits seen in a lesion with high grade dysplasia in BE

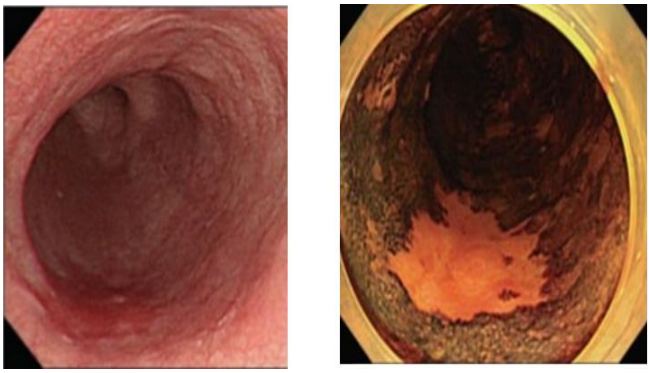

Figure 2: Lugol's iodine chromoendoscopy [21].
A) White light endoscopy presenting mucosa
B) Lugol's staining showing non uniform staining due to damaged epithelieum

6.1.3. Toulidine blue: Toluidine blue (also called tolonium chloride) is a basic dye that stains cellular nuclei. It is used to identify malignant tissues with high nuclear-to-cytoplasmic ratio, as in increased DNA synthesis. It can also selectively stain gastric cancers and provide better discrimination between benign and malignant ulcers [16, 17].

6.1.4. Cresyl violet: Cresyl violet solution (gentian violet) is an intra-vital stain used for the detection and characterization of early upper gastrointestinal cancers [18]. It is preferentially taken up in the crypts of Lieberkuhn, which appear as dots or pits, providing very clear definition of patterns having histological correlates.

6.1.5. Methylene blue: Methylene blue (MB) is the most commonly used vital stain, which is actively absorbed by mucosal tissues of small intestinal and colonic epithelium. The technique of methylene blue chromoendoscopy was originally described in 1979 by Japanese investigators, as a means for detecting intestinal metaplasia in the stomach [19]. Specialized columnar epithelium in Barrett's esophagus resembles gastric intestinal metaplasia, which is selectively stained by methylene blue as shown in (Figure 3). MB is taken up by

the intestinal epithelium after its local application in colon, resulting in a relatively stable staining pattern providing visualization of the openings of the glandular pits as shown in (Figure 4).

6.2. Reactive Stains

These stains undergo an observable change due to chemical process related to the function of gastrointestinal tract.

6.2.1. Congo red: Congo red CE can visualize the acid-secreting fundic mucosa. It changes the colour from red to black at a pH less than 3, in the presence of gastric acid. Hypo/achlorhydria is associated with H. pylori-induced or atrophic gastritis [24]. Staining pattern as observed with H. pylori infection is shown in as shown in (Figure 5).

6.2.2. Phenol red (PR): Phenol red detects alkaline pH by a gradual transition from yellow to red over the pH range of 6.8 to 8.2. The urease produced by the bacterium Helicobacter pylori catalyses hydrolysis of urea to NH_3 and CO_2 resulting in an increase in pH. As a result, H. pylori can be observed in red stained mucosa after phenol red CE as shown in (Figure 6).

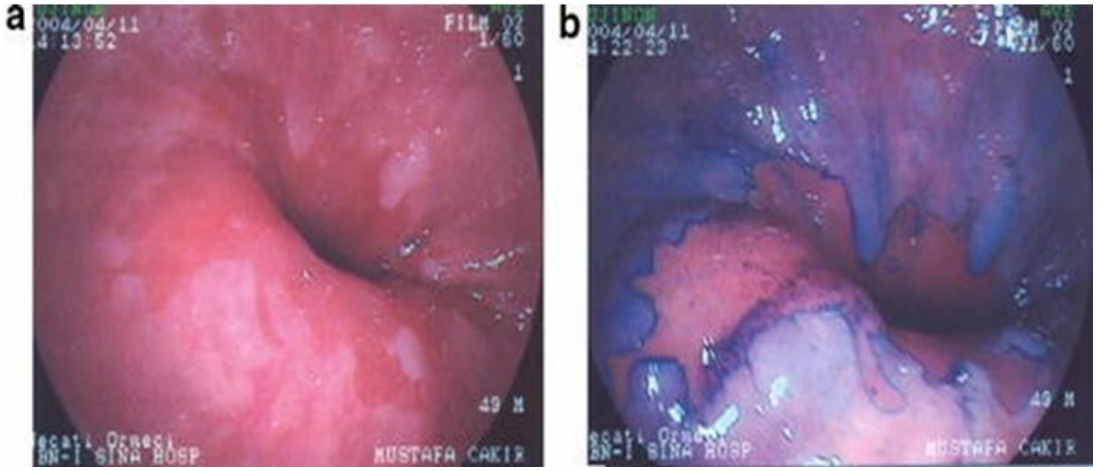

Figure 3: Methylene blue endoscopy of esophagus [22].
A) Conventional endoscopy in short-segment Barrett's esophagus.
B) CE with demarcation of short-segment Barrett's mucosa.

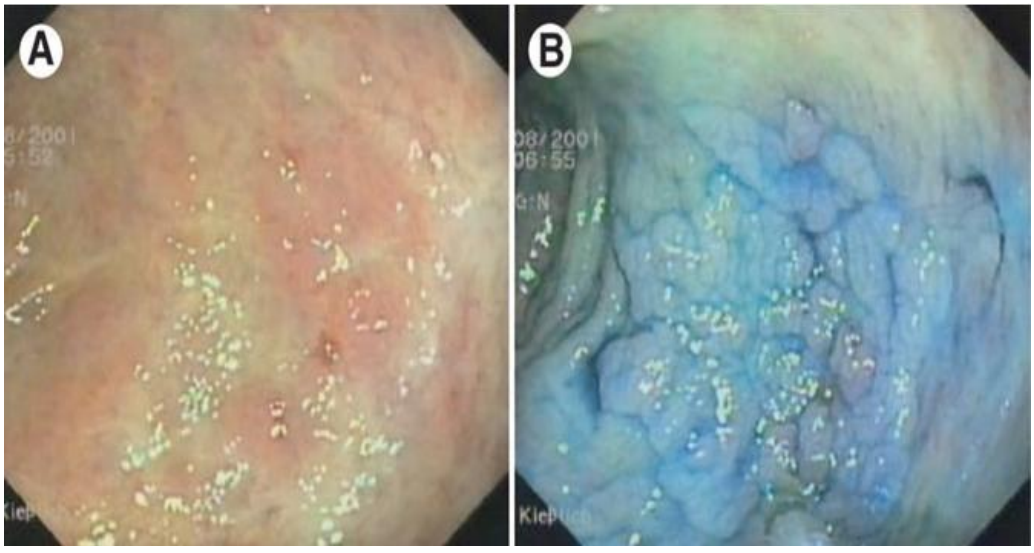

Figure 4: Methylene blue endoscopy of colon [23]
A) Colonic mucosa showing areas of focal erythema
B) MB chromoendoscopy showing intraepithelial neoplasia

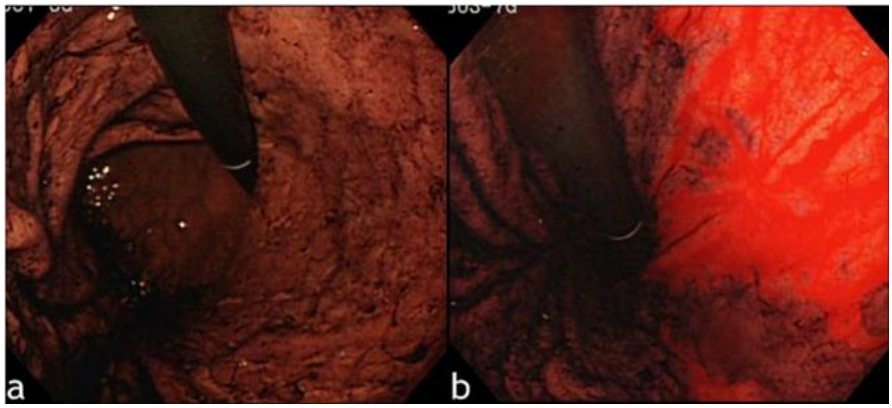

Figure 5: Congo red endoscopy of gastric region [25]
A) Black colour mucosa indicating no infection
B) Red colour mucosa showing *H. pylori* infection

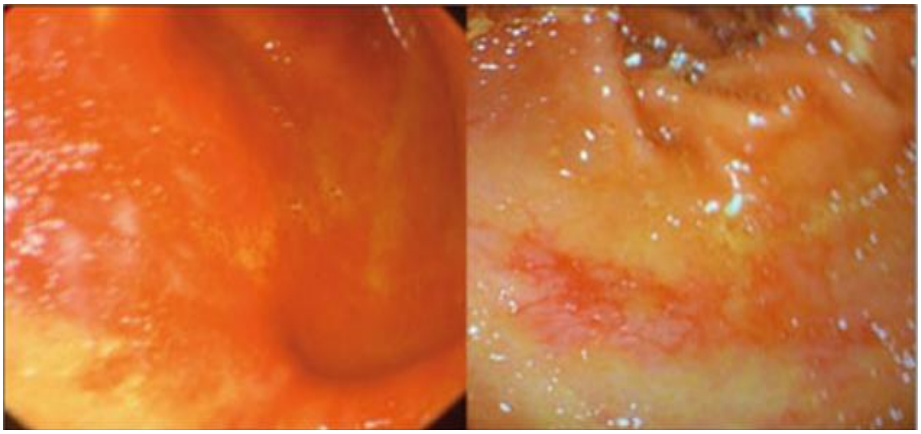

Figure 6: Phenol red endoscopy of gastric region [26]
A) Red stain indicating infection by *H. pylori*
B) Persisting yellow colour showing absence of infection

6.3. Contrast Stains

These are not absorbed but rather provide contrast by permeating between irregularities in mucosa to highlight the irregularities.

6.3.1. Indigo carmine (IC): This is the most commonly used stain with colonoscopy to enhance the detection of colorectal neoplasm. It is used for identification of dysplastic cells in individuals with chronic ulcerative colitis as shown in (Figure 7)**.** Due to the increased risk of dysplasia and colorectal cancer in patients with long standing IBD, endoscopic surveillance is strongly recommended. Currently, CE with targeted biopsies is the surveillance method recommended by the European Society of Gastrointestinal Endoscopy (ESGE) [27] and the British Society of Gastroenterology (BSG) [28].

7. Equipment

Generally, colouring agent is applied in solution form using a spray catheter. Spray catheter contains a tube carrying spray solution to which a metal tip is attached and carries nozzles. They are mostly disposable, flexible plastic sheaths with a metal nozzle tip. However, the channels of the equipment are to be cleansed with copious volumes of water during pre-cleaning procedure to avoid staining of unwanted regions. The amount of staining solution requires, depends on the surface area to be stained, but the smallest amount necessary should be applied to avoid dye pooling. In spray technique chances for homogenous distribution of dyes is less, which leads to non-uniform coating of mucosal areas and affects clinician's ability to assess lesions. Much of the stain is removed from the biopsy channel by suctioning water through the channel prior to routine cleaning which is a tedious procedure. Details of different spray catheters available globally are described in (Table 1) and corresponding images in (Figure 8).

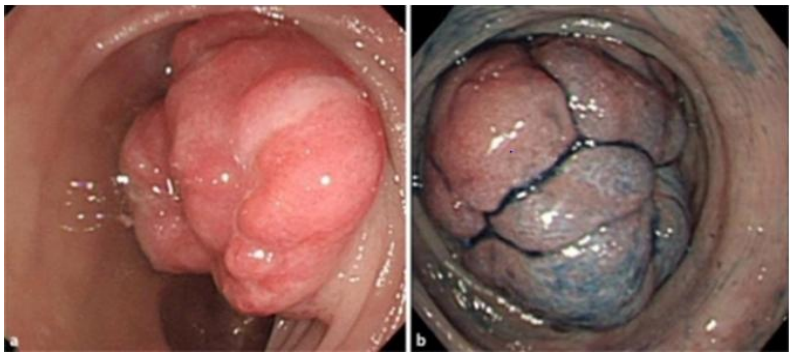

Figure 7: Indigo carmine CE in transverse colon [29]
A) Reddish and uneven lesion resembling type 1 tumour in colon
B) IC spraying showing clear borders of damaged tissue

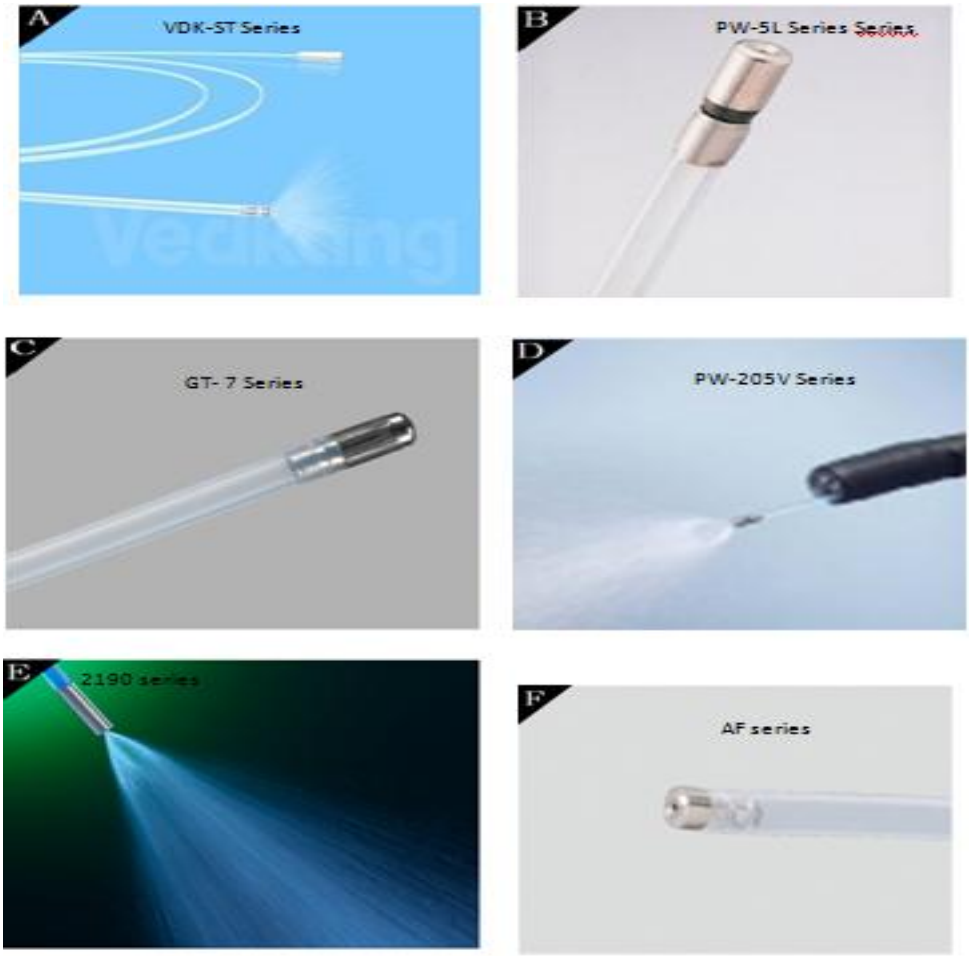

Figure 8: Images of spray catheters available globally .

8. Review on Clinical Trials Using Dyes

A detailed review is presented below in (Table 2) explaining the methods and results obtained in various endoscopic procedures using dyes.

9. Conclusion

From the above presented data, it can be clearly confirmed that CE is an accurate, simple, safe, and reproducible method for detecting certain GI abnormalities associated with the use of spray catheters. However, the use of a spray catheter has certain limitations like cost, suction capacity of the endoscope and often requires several passages with the endoscope, which can sometimes be difficult in some patients. Damage to the tissue by spay catheter is a major problem associated with traditional spraying technique. Lag time may not be

enough after staining for simultaneous observation while staining. This may cause non uniform dye distribution and can lead to improper interpretation of results. Tissue folds occurring in stomach or small intestine and folds which may develop due to inherent disease conditions can also obstruct the dye spraying and distribution. To overcome the above discussed disadvantages, alternative methods of dye instillation can give better and superior results without economic concern.

10. Acknowledgments

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Table 1: Description of globally available spray catheters.

Label	Name	Product Code	Specifications	Major Market	Ref No.*
A	Single use spray catheter from Vedkangmed	VDK-ST Series	2300mm in length and 2.3mm diameter or 1800mm in length and 1.8mm diameter	China	30
B	Re usuable spray catheter from Olympus.	PW-5L Series	Working Length: 1650mm Minimum working channel: 2.8mm	Australia	31
C	Glo-Tip® single use spray catheter from cookmedical.	GT- 7 Series	Catheter length 240cm, Stylet length 100cm	United States	32
D	Disposable spray catheter from Olympus.	PW-205V Series	2400 mm in length 2.45 mm insertion pore diameter	Australia	33
E	Hobbs medical Disposable spray catheter	2190 and 2191	2.6mm in diameter and 2 working lengths 260cm and 165cm	United States	34
F	Omni-med disposable spray catheter	AF Series for gastroscopy and colonoscopy	2.8mm diameter and 1800 or 2300mm in length	United Kingdom	35

Table 2: Literature review on dyes used in CE.

Dye	Study/Methods	Results/Inference	Ref.
1. Absorptive or vital stains			
Acetic acid	Standard endoscopy was followed by magnification endoscopy and procedure was repeated after 1.5% acetic acid spraying on 49 patients undergoing endoscopic surveillance because of short segment BE without dysplasia.	Acetic acid magnification endoscopy is a simple technique to identify characteristic endoscopic mucosal surface patterns with outstanding clarity and resolution that correlate with histologic identification of specialized intestinal metaplasia	36
	Inspection of Barrett's neoplasia was carried out using white light followed by acetic acid on 197 patients. 263 procedures were performed on to examine the efficacy and potential cost implications of acetic acid CE in the assessment of Barrett's neoplasia.	High risk neoplasia was found on 143 procedures. 55% of cases were only detected with white light endoscopy, whereas, by using acetic acid it was correctly identified in 96% of the cases. This potentially represented a significant cost saving in patients with suspected neoplasia	37
	To investigate the effectiveness of acetic acid CE in a BE surveillance, a retrospective study was carried on 655 subjects.	Acetic acid detected more neoplasias than conventional protocol-guided mapping biopsies and required 15 times fewer biopsies per neoplasia detected.	38
	To compare acetic acidbased CE with histopathology as the reference standard a meta-analysis was performed on 1379 subjects for detecting early neoplasia in patients with BE and 516 subjects for specialized intestinal metaplasia.	Acetic acid has an overall high diagnostic accuracy for detecting early neoplasia in patients with BE. For specialised intestinal metaplasia characterization, sensitivity is very high but has poor specificity, suggesting that histological confirmation is necessary when it's positive.	39
Lugol's iodine	124 subjects were endoscopically examined using 5% Lugol's solution on esophageal epithelium.	5% Lugol's solution was useful for differential diagnosis of various esophageal diseases, esophagitis, esophageal cancer, submucosal tumour of esophagus and hiatus hernia.	15
	A prospective study involving 60 patients was carried out to screen for superficial esophageal cancer and dysplasia using endoscopy and a 2% lugol dye solution followed by biopsy of the suspicious areas.	Superficial esophageal cancer is extremely difficult to detect by conventional methods in asymptomatic patients. Endoscopic screening of the esophagus using lugol dye in patients can provide early prognosis.	40
	64 patients who underwent EGD for esophageal squamous cell carcinoma screening were studied to clarify the endoscopic mucosal change of the stomach caused by Lugol's iodine solution spray.	In 51 patients (80%) mucosal change was observed as as fold thickening, and reticular pattern of white lines was found on the surface of the thickened gastric folds in 28 of the patients (44%). Exudates were observed in 6 patients (9%). From the above data use of Lugol's solution for determination of mucosal change was confirmed and it should be noted that gastric fold thickening, should not be misdiagnosed as a severe gastric disease.	41
	A total of 15,264 residents in rural Hua County, were screened by Lugol CE. Biopsies were collected from endoscopically visualized lesions, identified before and after Lugol CE and analyzed histologically.	586 participants were found by biopsy analysis to have esophageal squamous dysplasia or more severe lesions. Lugol CE sensitivity values for the detection of mild, moderate, and severe dysplasia, and esophageal cancer, were 45.9%, 55.3%, 87.0%, and 97.7%, respectively and CE with Lugol solution can be used to identify patients with esophageal cancer with almost 98% sensitivity.	42

Toluidine blue	280 biopsy-proven oral squamous cell carcinoma margins were analyzed using toluidine blue staining and frozen section histopathology	Toluidine blue staining had sensitivity and specificity of 100% and 97%, respectively. The diagnostic accuracy of toluidine blue was found to be 97.1% and can be concluded that toluidine blue can be used as an effective screening modality for the assessment of intraoperative margins in resource limited environments and reducing the number of frozen section biopsies performed.	43
	Chemiluminescent illumination, toluidine blue supravital staining, oral exfoliative cytology and biopsy were performed on 3 groups. Group I consisted of normal appearing mucosa. Group II and III consisted of clinically diagnosed pre-cancer and clinically suggestive of cancer mucosa.	Toluidine blue staining test was reliable in precancerous and cancerous lesions, which was presented as erosive and red-white lesions. It showed negative result to keratotic lesions.	44
Cresyl violet	To establish the staining characteristics and optimal concentration of cresyl violet, the ileum and colon of 7 BL6 mice were stained with CV (0.1%-2%), and *in vivo* confocal imaging was performed	Cresyl violet can be applied topically and allowed simultaneous chromoendoscopy and endomicroscopy with accurate prediction of histology with visualization of nuclear morphology.	45
Methylene blue	Upper endoscopy with methylene blue-directed biopsy was obtained from 14 patients with BE and 12 control patients. Biopsy specimens were independently examined by two pathologists unaware of the endoscopic results.	The overall accuracy of methylene blue staining for detecting specialized columnar epithelium was 95% and procedure was reported to be safe, inexpensive, reproducible, and highly accurate method for diagnosis of specialized columnar epithelium in BE.	46
	Chromoendoscopy of the distal esophagus with 1% methylene blue was performed on a total of 73 patients to obtain targeted biopsy	The targeted biopsy of stained areas provided histologic proof of specialised columnar epithelium with a sensitivity of 98% and a specificity of 61%. MB staining increased the rate of detection of specilised columnar epithelium, both in patients with visible columnar lined distal esophagus and with normal gastresophageal junction.	47
	In vivo and *ex vivo* study was performed to evaluate methylene blue staining properties of dysplastic and nondysplastic BE.	The accuracy of *ex vivo* and in *vivo* methylene blue staining for specialized columnar epithelium was 87% and 90%, respectively which confirmed the staining properties of methylene blue.	48
	297 biopsy specimens from 30 patients with long-segment BE were obtained from areas that stained positive and negative with methylene blue.	From sensitivity, specificity, and negative and positive predictive values it can be summarised that methylene blue CE can be used for detection of specialized intestinal metaplasia in long-segment BE, but not in short-segment BE.	49
	Prospective, randomized crossover study comparing 4-quadrant random biopsies (4QB) versus MB-directed biopsies for the detection of Specialised Intestinal Metaplasia was evaluated in 48 patients with long segment Barrett's esophagus	The mean number of biopsies taken during 4QB was 18.92 +/- 6.36 and with MB was 9.23 +/- 2.89. The sensitivity of MB for SIM and dysplasia was 75.2% and 83.1%, respectively. The yield of 4QB for identifying non-dysplasia SIM was 57.6% and for dysplasia was 12%. Results concluded that MB requires significantly fewer biopsies than 4QB to evaluate for SIM and dysplasia.	50
	To evaluate the effectiveness of methylene blue–targeted biopsies in the differential diagnosis of intestinal metaplasia, dysplasia, and superficial esophageal carcinoma, a clinical trial was performed on a total of 109 patients	CE is useful for delineating Barrett's epithelium and for indicating the correct location for securing biopsies where dysplasia or early esophageal cancer is suspected.	22
2. Reactive stains			
Congo red	About 100 outpatients with or without dyspeptic symptoms referred for endoscopy were evaluated by using a modified endoscopic congo red test and a standard test of gastric acid secretion	Results indicated thatmodified endoscopic congo red test as an accurate, simple, fast, inexpensive, and well-tolerated chromoendoscopic method for identification of patients with hypo/achlorhydria during routine upper endoscopy.	51
Phenol red	Analytical study on sample constituted by 195 patients for detection of *Helicobacter pylori* infection.	Results indicated that sensibility, specificity and exactitude in diagnosis with phenol red at 0.1%(w/v) concentration was over 70.0%.	52
	Correlation of PR staining in urea breath test (^{13}C-UBT) was studied on 123 patients.	Extent of staining, expressed as a staining score, was positively correlated with the change in ^{13}C-UBT. The pH of stained mucosa was significantly higher than that of unstained mucosa.	53
	80 subjects were studied to compare, the diagnostic value of *H. pylori* detection using phenol red chromo-endoscopy, and rapid immunoassay in stool specimens (Rapid Strip HpSA®.). Histopathology, the gold standard, was taken as reference.	Results indicated that phenol red CE was more specific and less sensitive than the rapid stool Rapid Strip HpSA® test for detection of *H. pylori* infection. Advantages included immediate reading, ability to detect focal and scattered infection which can be missed by random biopsy taking.	54
3. Non-absorptive stains			
Indigo carmine	As depressed early cancers and flat adenomas have a high potential for malignancy with possible infiltrating growth, the usefulness of chromoendoscopy during routine colonoscopy using vital staining with indigo carmine solution (0.4 %, 1 - 10 ml) on all visible lesions was performed in 100 consecutive patients .	Each lesion was classified according to type (polypoid, flat, or depressed), position and size. The staining pattern was classified according to the pit pattern classification which allowed differentiation between hyperplastic and adenomatous lesions. This technique unmasked multiple mucosal lesions which were not identified by routine video colonoscopy. The pit pattern seen after staining allowed differentiation between hyperplastic and adenomatous lesions required for endoscopic interventions.	55
	A prospective study to determine the effect of indigo carmine dye spraying with and without magnification endoscopy was carried on 122 patients.	Diagnostic accuracies for conventional, dye based and magnified chromoendoscopy are 84%, 89.3% and 95.6% respectively. CE with magnification was reported as the most reliable method for determining whether a colorectal lesion is non-neoplastic or neoplastic.	56
	Aprospective, randomised two-centre trial, with 0.4% indigo carmine spraying during continuous extubation was carried and compared with standard colonoscopy (control group) in consecutive patients attending for routine colonoscopy in about 1000 patients.	Adenoma detection rates in an average-risk population using indigo carmine is practicable enough for routine application as there was stastically significant increase in detection of flat adenomas as well as serrated lesions.	57
	316 patients were divided into two groups as control and CE group. In the control group, the ascending colon and cecum were observed twice without CE. In the CE group, the cecum and ascending colon were re-inspected following staining with indigo carmine solution after conventional examination of the cecum and ascending colon.	Multivariate analysis revealed that detection of polyps after indigo carmine spraying was independently associated with a high body mass index and older age. The CE group differed significantly from the control group in the number of additionally detected polyps and can be concluded that using CE with indigo carmine polyps detection is easy and reliable.	58
	To compare the accuracy of digital and real-time chromoendoscopy for the differential diagnosis of diminutive (<5 mm) neoplastic and non-neoplastic colorectal lesions, a prospective randomized study analyzed 215 colorectal lesions less than 5 mm in 134 patients.	Pit pattern analysis with indigo carmine and magnification showed 96.5% sensitivity, 88.2% specificity, and 94.2% accuracy for the same purpose. Both magnifying virtual chromoendoscopy and indigo carmine chromoendoscopy showed high accuracy in the histopathological diagnosis of colorectal lesions less than 5 mm in diameter.	59
	Effectiveness of indigo carmine based chromoendoscopy for neoplasia detection and characterisation, in real-life was studied in 350 subjects in a multicentre cohort study.	Specific lesions features were recorded, and optical diagnosis was assessed between expert and non-expert endoscopists. The study concluded showing high diagnostic yield for neoplasia detection and lesion characteristics were helpful to aid the endoscopist for *in situ* therapeutic decisions.	60

	To compare the effectiveness of *in vivo* diagnosis of small colonic polyps using indigo carmine dye spray with standard-definition and high-definition colonoscopes 237 polyps were evaluated and predicted diagnosis was compared with the true histology.	The accuracy of *in vivo* assessment of small colonic polyps with indigo carmine dye spray was excellent with standard-definition colonoscopes and is not improved with high-definition colonoscopes as there was no statistically significant difference for any of the measured parameters.	61

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