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Esophageal dysplasias are detected by endoscopy with Lugol in patients at risk for squamous cell carcinoma in southern Brazil

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SUMMARY. **Diagnosis of squamous cell carcinoma of the esophagus is usually late. Staining of the mucosa with Lugol's solution during endoscopy has been suggested to identify early cancer/dysplasia and may improve prognosis. Lugol was tested during endoscopy in 96 asymptomatic subjects at risk for this tumor, who were found to have atypias after exfoliative cytology in southern Brazil. Biopsies were obtained in Lugol's 'stained' and 'unstained' areas in the esophageal mucosa and the histologic results were compared. 'Unstained' areas were present in 64 (66.7%) instances: 44 'unstained' areas over mucosa with normal appearance revealed seven dysplasias (four high and three low grade), whereas 20 'unstained' areas with visible lesions contained only one dysplasia (low grade). 'Stained' areas in 96 (100%) subjects showed two additional dysplasias (one high and one low grade). In this study, Lugol 'unstained' areas were of great value for detection of dysplasias (sensitivity = 80%; specificity = 63%; p = 0.01, Fisher's exact test; CI = 95%; odds ratio = 6.7).**

INTRODUCTION

Squamous cell carcinoma of the esophagus (SCCE) is one of the most common tumors, especially in China, Iran and in certain republics of Central Asia, a region known as the 'Asian belt of esophageal cancer'. In this region SCEE has a prevalence of more than 100 per 100 000 among both sexes.[1,2] In southern Brazil, in the state of Rio Grande do Sul, SCCE is the fourth most frequent neoplasm in men and the eighth most common in women, and in certain areas, such as in the city of Taquara, its prevalence may reach up to 29.4 and 10.4 per 100 000 respectively.[3–5] It represents more than 85% of all cases of esophageal cancer in our hospital.[6] Diagnosis and treatment are usually late and carry a poor prognosis.[7–9]

In 1933, Schiller used Lugol solution for the diagnosis of minute uterine cervical carcinoma, but only in 1966 was Lugol introduced for endoscopic examinations of the esophagus.[10,11] It has been proposed, recently, as an important diagnostic technique for early detection of SCCE or its precursor lesions in the form of dysplasias.[8,9,12,13] Iodine contained in the Lugol solution reacts with glycogen contained in normal squamous epithelium, staining the esophageal mucosa chestnut brown. Abnormal areas of these epithelia exhibiting chronic inflammation, dysplasias or cancer do not contain glycogen and are not colored by Lugol solution.[13–15] The objective of the present study was to evaluate the diagnostic value of endoscopy with Lugol spraying of the esophageal mucosa to detect dysplasia or early cancer in individuals at risk for SCCE in southern Brazil.

METHODOLOGY

The sample population of the study was composed of male and female inhabitants of the city of Taquara who volunteered for a screening program involving an esophageal exfoliative cytology examination using a model of a cytological balloon developed in the Bioengineering Laboratory of the Hospital de Clínicas

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de Porto Alegre. This technique allows detection of suspected cytological abnormalities such as cancer or dysplasia (presence of macronuclei, nucleus presenting dysplastic chromatic alterations, multinucleations with nuclear atypias, dyskeratosis). Individuals answered a questionnaire designed to evaluate risk factors for SCCE and were excluded if they presented any of the following symptoms: progressive dysphagia, upper digestive tract hemorrhage in the previous 30 days, history of coagulation disorders, ascites, jaundice, severe arterial hypertension and encephalopathy of any nature. All individuals signed an informed consent approved by the Hospital's Ethics Commission according to the Helsinki declaration for participation in the research protocol.

The endoscopies were always performed by at least two experienced gastroenterologists (S. G. S. Barros, C. P. F. Freitag, H. P. Breyer). The examination began after topical anesthesia with xylocaine spray to the oropharynx and sedation with intravenous midazolam with the individual lying in a left lateral recumbent position. The oropharynx was examined first, followed by the esophagus in a to-and-fro fashion. Notes were taken as to any irregular area in the mucosa (erosion, red focal area, plaque, nodule) in the conventional examination as previously described.[16] Next, the stomach and the duodenum were evaluated. After removal of esophageal secretions with water, the organ was sprayed with 10–15 ml of 3% Lugol solution (12 g iodine + 24 g potassium iodide in 1000 ml water) with an Olympus catheter model PW-52. Notes were also taken as to the presence and location of 'unstained' areas and their coincidence with visible lesions observed during conventional endoscopy. We considered the presence of visible lesions when coincident with 'unstained' areas as suspicious for dysplasia and/or early cancer. Biopsies were taken of the 'unstained' areas and two or three fragments were collected. When more than one 'unstained' area was found, the clearest and brightest one was biopsied after agreement by the two examiners. Two or three biopsies were also obtained in homogeneously stained areas at least 2 cm away from the 'unstained' areas. When there were no 'unstained' areas, the middle third of the esophagus was regularly biopsied. The specimens obtained were fixed in 10% formaldehyde solution and sent to the pathology unit.

The average time for the endoscopic examination with Lugol was calculated and compared with the average duration of 100 consecutive, routine upper gastrointestinal endoscopies performed by the same examiners at the same unit.

The alterations observed in the histopathological examination, according to previously published criteria,[13,17] were classified as follows: normal; mild chronic esophagitis; moderate chronic esophagitis, severe chronic esophagitis; 'low-grade' dysplasia; 'high-grade' dysplasia; invasive or superficial carcinoma; insufficient or inadequate material. The significance in the association of variables was determined by Fisher's exact test and the odds ratio with a confidence interval of 95%.

A sample was randomly selected for verification of concordance (kappa test) between two independent pathologists and consisted of the following: seven biopsies diagnosed as normal epithelium or mild chronic esophagitis, seven as moderate/severe chronic esophagitis, and seven as dysplasia/early cancer. All dysplasias were revised, and the diagnosis 'dysplasia' or early cancer was only given when there was a diagnostic agreement.

RESULTS

Between June 1994 and January 1997, 1160 people underwent esophageal exfoliative cytology with the cytological balloon. Of these, 112 had suspicious alterations and were referred for upper gastrointestinal endoscopy. Ten people were excluded from the study: five who did not come to the examination, four as a result of psychomotor agitation after the injection of midazolam, which prevented the continuation of the examination, and one because of insufficient material for histopathological evaluation. Additionally, six individuals were excluded from the analysis because they showed advanced neoplastic lesions with maximum diameter varying between 2 and 5 cm. These tumors involved the middle and/or the distal third of the esophagus. When asked again after the endoscopy, these individuals admitted to occasional dysphagia; however, this was not progressive and was without significant weight loss. Subjects with tumors were directed toward surgical and/or radiotherapy treatment. These six subjects were excluded because Lugol was being tested for the detection of dysplasias or early cancer that have a better prognosis and are difficult for the endoscopist to visualize.[7,9,13,16]

The average time for upper gastrointestinal endoscopies with Lugol spraying and collection of biopsies was 20 min, whereas that for routine upper gastrointestinal endoscopies in the control group was 12 min.

The population effectively studied consisted of 96 patients. Fifty-nine patients were men (61.5%), and they were on average 57.9 years of age ($\pm$11.6). As to risk factors among the patients, 87 (90.6%) drank hot tea (maté), while 53 (55.2%) smoked and 22 (22.9%) drank alcoholic beverages daily. Weekly heartburn or odynophagia was present in 34 (35.4%) individuals. Conventional endoscopy revealed visible lesions in the esophageal mucosa in 64 patients (66.7%), but only 20 appeared as 'unstained' areas after the Lugol spraying. Among these 20 areas biopsied, only one contained a low-grade dysplasia (Tables 1 and 2).

Table 1. Findings at conventional esophagoscopy and the mucosal pattern after Lugol spraying (n = 96)

	Mucosal pattern after Lugol spraying			
		'Unstained'		
Conventional esophagoscopy	'Stained' [n (%)]	Coincident[a] [n (%)]	Not coincident[b] [n (%)]	Total (n)
Visible lesions	18 (56.3)	20 (100)	26 (59.1)	64
Erosion	10 (31.2)	14 (70)	14 (31.9)	38
Red focal area	–	1 (5)	–	1
Plaque	1 (3.2)	1 (5)	–	2
Nodule	1 (3.2)	2 (10)	2 (4.5)	5
Others[c]	6 (18.7)	2 (10)	10 (22.7)	18
No visible lesions	14 (43.7)	–	18 (40.9)	32
Total	32 (100)	20 (100)	44 (100)	96

(a) 'Unstained' areas co-incident with visible lesions through conventional upper gastrointestinal endoscopy; (b) 'Unstained' areas not co-incident with visible lesions through conventional gastrointestinal endoscopy; (c) Others, small red islands near Z line, depression, ulceration, scattered whitish plaques (confirmed by microscopic direct examination as *Candida* sp.), hiatal hernia, hyperemia distal esophagus.

Table 2. Histologic diagnosis in 'unstained' areas after Lugol (n = 64)

	Histologic diagnosis								
	Areas coincident with visible lesions				Areas not coincident with visible lesions				
	Dysplasia [n (%)]				Dysplasia [n (%)]				
Conventional esophagoscopy	HG	LG	Esophagitis [n (%)]	Normal [n (%)]	HG	LG	Esophagitis [n (%)]	Normal [n (%)]	Total (n)
Visible lesions	–	1 (100)	17 (100)	2 (100)	2 (50)	1 (33.3)	12 (63.2)	11 (61.1)	46
Erosion	–	1 (100)	12 (70.5)	1 (50)	1 (25)	1 (33.3)	6 (31.6)	6 (33.3)	28
Red focal area	–	–	1 (5.9)	–	–	–	–	–	1
Plaque	–	–	1 (5.9)	–	–	–	–	–	1
Nodule	–	–	2 (11.8)	–	–	–	2 (10.5)	–	4
Others[a]	–	–	1 (5.9)	1 (50)	1 (25)	–	4 (21.1)	5 (27.8)	12
No visible lesion	–	–	–	–	2 (50)	2 (66.6)	7 (36.8)	7 (38.9)	18
Total	–	1 (100)	17 (100)	2 (100)	4 (100)	3 (100)	19 (100)	18 (100)	64

HG, high-grade dysplasia; LG, low-grade dysplasia; (a) others, small red islands near Z line, depression, scattered whitish plaques (confirmed by microscopic direct examination as *Candida* sp.), hiatal hernia, hyperemia distal esophagus.

After Lugol spraying in 96 patients, 'unstained' areas were present in 64 (66.7%) and were located in the distal esophagus in 42, in the middle esophagus in 21, and in the proximal esophagus in one. Small 'unstained' or poorly stained areas were also seen in these patients but were not biopsied according to our protocol. The mucosa appeared normal at the time of the conventional endoscopy (and after videotape review) in 44 (69%) of these 'unstained' areas. Histopathological examination of biopsies from the 'unstained' areas showed the following results: 'normal' in 21 (32.8%) patients, 'mild, moderate or severe esophagitis' in 35 (54.7%), and 'dysplasia' in seven (10.9%) patients, of which four were high-grade and three were low-grade dysplasias (Table 3). Biopsies of areas uniformly 'stained' from Lugol in the 96 patients revealed the following results: 'normal' in 44 (45.8%) patients, 'chronic esophagitis' in 50 (52.1%), and 'dysplasia' in two (2.1%), of which one was high grade and one was low grade (Table 3; Fig. 1). Early cancer was not found.

The sensitivity, specificity, and predictive positive and negative values of 'unstained' areas after Lugol staining to detect dysplasias were compared with the findings in the 'stained' areas (Table 4). Sensitivity

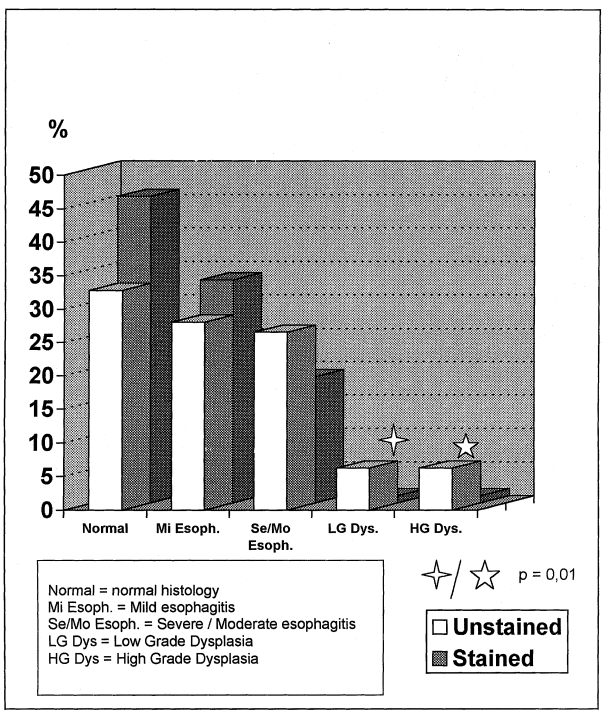

Fig. 1—Histopathologic findings in unstained and stained areas (n = 160).

Table 3. Histologic diagnoses in 'unstained' and 'stained' areas by biopsy sites (n = 160)

Histopathological findings	'Unstained' areas [n (%)]	'Stained' areas [n (%)]	p*
Normal	21 (32.8)	44 (45.8)	NS
Mild esophagitis	18 (28.1)	32 (33.3)	NS
Moderate/severe esophagitis	17 (26.6)	18 (18.8)	NS
Low-grade dysplasia	4 (6.25)	1 (1.05)	0.01
High-grade dysplasia	4 (6.25)	1 (1.05)	0.01
Total	64 (100)	96 (100)	

*Fisher's exact test.

Table 4. Determination of sensitivity, specificity, and positive and negative predictive values of 'unstained' areas for detection of dysplasias according to biopsy sites (n = 160)

	Dysplasia		
	Positive	Negative	Total
'Unstained' areas	8*	56	64
'Stained' areas	2	94	96
Total	10	150	160

Sensitivity = 80%; specificity = 63%; positive predictive value = 12,5%; negative predictive value = 98%; *p = 0.01 (Fisher's exact test). No cases of early cancer were found.

was 80% (95% CI = 71–89%), specificity was 63% (95% CI = 56–70%), positive predictive value was 12.5% (95% CI = 7.5–17.5%), and negative predictive value was 98% (95% CI = 96–100%). The odds ratio for 'unstained' areas to present dysplasia in comparison to stained areas was 6.7 (95% CI = 1.3–6.3). The concordance between the pathologists was moderate (kappa = 0.41).

DISCUSSION

Survival rates for squamous cell carcinoma of the esophagus are low in southern Brazil because of late diagnosis. Early detection seems to be the best or perhaps the only chance of improving the efficacy of treatment for SCCE allowing for higher survival rates.[7] Emphasis must be placed on the detection and removal of early cancer or its precursor lesion dysplasia.[18,19] Dysplasia and SCCE are known to occur in a multicentric fashion and there is a definite continuity between high-grade dysplasias and carcinoma.[20,21] Dysplasias or early cancer are occasionally described as causing intermittent dysphagia or no symptoms at all, rarely raising a clinical suspicion for early cancer.[21] Also, alterations in the mucosa which are indicative of dysplasia and/or carcinoma restricted to the mucosa are difficult to diagnose through conventional endoscopy.[7,9,14]

The use of Lugol solution in upper gastrointestinal endoscopy has been suggested as increasing the sensitivity of the examination for detection of dysplasias and/or early cancer, but studies in Western populations are scanty and much needed.[9,13,15,22–26] The screening program was initiated with inhabitants of the city of Taquara in southern Brazil who volunteered to test Lugol spraying during endoscopy for diagnosis of dysplasias.

The majority of the 96 individuals studied were men older than 44 years of age who were exposed to known risk factors in that population, such as smoking and consumption of hot maté and alcoholic beverages daily.[27,28] Incidentally, the frequent findings of weekly heartburn and/or occasional odynophagia and the presence of linear erosions in the third distal of the esophagus in over one-third of the individuals suggest the probability of significant gastroesophageal reflux in that population. Performing endoscopy with Lugol was considered easy by the examiners and on average took only an additional 8 min when compared with routine upper gastrointestinal endoscopies in the control group.

The frequency of dysplasias in the group studied was increased (10.4%) and dysplasias occurred mainly in men. Of the 64 areas 'unstained' by Lugol, 20 occurred in sites with lesions (erosion, red focal area, plaque and nodule) visible on conventional endoscopy, previously described as suspected of exhibiting dysplasia or cancer in Chinese subjects.[16] Nonetheless, biopsies of those 20 areas revealed only one low-grade dysplastic lesion. The other 44 areas that were 'unstained' after Lugol spraying occurred in mucosa that appeared normal and apparently innocent at the time of conventional endoscopy and disclosed seven dysplasias, four of which were high grade. Thus, the great majority of dysplasias detected were found in mucosa that appeared to be normal and were only disclosed after the spraying of Lugol. 'Unstained' areas had approximately a seven times greater likelihood (OR = 6.7, p = 0.01) of exhibiting dysplasias than 'stained' areas. The high negative predictive value (98%) found suggests that individuals with mucosa homogeneously stained after Lugol spraying have a low probability of dysplasias. A follow-up study of those 96 individuals is now under way and should further evaluate the spraying of Lugol as a diagnostic tool.

In this study, the presence of visible lesions during conventional endoscopy that were 'unstained' after Lugol had a low probability for containing dysplasias. These data are different from those observed by Dawsey et al[13,16] in a larger study with Chinese subjects in a much higher risk area for this cancer, when visible lesions (erosion, red focal area, plaque and nodule) had a high positive predictive value for detection of dysplasias and cancer. In the present study, we may have had a selection bias in that we ignored visible lesions, when stained by Lugol, and also small 'unstained' areas, both of which were not biopsied according to our original protocol. These non-biopsied areas could also contain dysplasias or

even cancerous lesions. Further studies with biopsies of all unstained and all visible lesions must be carried out, especially in Western populations.

In this study, Lugol was valuable for the detection of dysplasias and should be considered as an important addition to the upper gastrointestinal endoscopy in subjects at risk (smokers, alcohol drinkers, hot tea consumers) for squamous cell carcinoma of the esophagus.

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