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The evaluation of Lugol's iodine solution staining combined with endoscope for diagnosis of non-erosive reflux disease in clinic

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Abstract. – AIM: To evaluate the value of Lugol's iodine solution staining combined with endoscope on the diagnosis of non-erosive reflux disease (NERD).

PATIENTS AND METHODS: A total of 96 gastroesophageal reflux disease patients were selected to participate in this study. The patients were stained on esophageal mucosa by Lugol's iodine solution and examined at routine endoscopy. The shallow staining and/or non-staining group patients were treated with esomeprazole and mosapride citrate, and then the changes in Lugol's iodine staining, Gerd Q (Gerd questionnaire) scoring and histological characters of esophageal mucosa were recorded before and after treatment.

RESULTS: As the results, a total of 68 patients were diagnosed as NERD, and 36 of 68 patients were observed with uniform staining and 32 of 68 patients were observed with shallow staining and/or non-staining. After 4 weeks for treatment, 28 of 32 patients with shallow staining and/or non-staining became uniform staining and 4 of 32 patients were still with shallow staining and/or non-staining. Before and after treatment, the Gerd Q scoring of uniform staining groups and shallow staining and/or non-staining groups all had a significant difference ($p < 0.05$). Compared to the routine endoscopy, the detection rate of abnormal esophageal mucosa was significantly increased by Lugol's iodine solution staining combined with endoscope.

CONCLUSIONS: Lugol's iodine solution staining combined with routine endoscopy, Gerd Q scoring and histomorphology can be used to evaluate the diagnosis and therapeutic effect of NERD.

Key Words:
Endoscope, Gerd Q score, Lugol's iodine solution staining, Non-erosive reflux disease.

Introduction

Among gastroesophageal reflux disease (GERD) patients, those with heartburn and acid regurgitation symptoms but are not examined esophageal mucosal injury at routine endoscopy, namely non-erosive reflux disease (NERD) patients[1]. Recently, the NERD is diagnosed in clinic not only by medical history and questionnaire but also advanced endoscopic techniques such as 24-h pH monitoring, high resolution and high definition white light endoscopy, chromoendoscopy, magnification endoscopy, narrow-band imaging, confocal laser endomicroscopy and high definition endoscopy with I-scan[2-5]. However, the lack of advanced equipment, the complex of operation, the high cost of examination and other factors limit these technologies are widely used in the primary hospital[4,6]. Therefore, in clinical practice, the management of NERD patients is still with challenging.

Recently, double vital staining with Lugol's iodine and methylene blue have been reported in diagnosing superficial esophageal lesions[7] and Lugol-combined endoscopy with biopsy has been proven to be the most effective technology for detecting the presence and spread of small malignant lesions of the esophagus[8]. In this study, for the NERD patients treated before and after with esomeprazole and mosapride citrate, we used Lugol's iodine solution staining with sensitivity at endoscope to evaluate the changes in staining, Gerd Q (Gerd Questionnaire) score and the histomorphology and explored the effectiveness of vital staining with Lugol's iodine solution combined with routine endoscopy for NERD diagnostic.

Patients and Methods

Study Design

The study design for this paper was shown in Figure 1 (see Figure 1).

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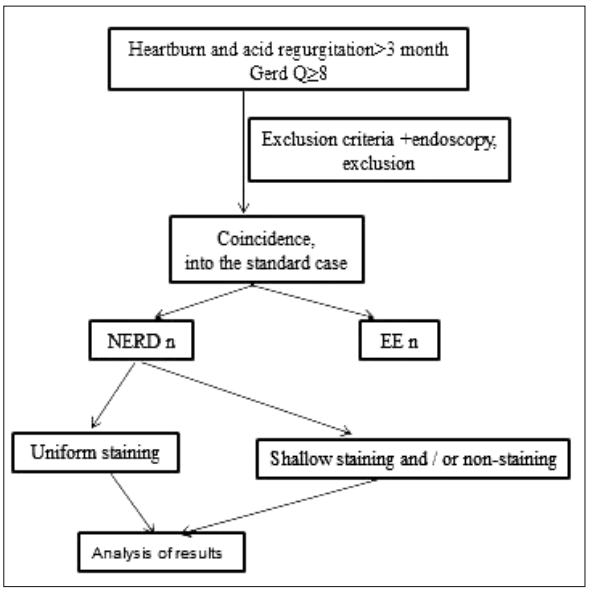

Figure 1. Study design.

Patients' Selection

The GERD patients with heartburn and acid regurgitation but no exhibiting esophageal mucosal injury at routine endoscopy, Gerd Q ≥8, and presenting in Gastrointestinal Clinic of Qingdao Central Hospital from June 2010 to June 2012 were invited to participate in this study. This study was supported by the Ethics Committee of Qingdao Central Hospital.

The exclusion criteria were: (1) Patients with serious heart, liver, kidney, lung disease. (2) Patients with digestive tract cancer, peptic ulcer disease and gastrointestinal surgery. (3) Women during lactating and pregnancy. (4) Patients with alarm symptoms such as vomiting, weight loss, difficulty in swallowing, hematemesis, black stools, anemia and others. (5) Patients had taken proton pump inhibitors in the past 4 weeks. (6) Patients with scleroderma. (7) Patients had allergies to iodine or refuse to iodine staining. (8) Patients with history of hyperthyroidism. (9) Patients with history of drug abuse. (10) Patients with esophageal mucosal injury at routine endoscopy and would be diagnosed as erosive esophagitis (EE).

Endoscopy and Staining

All selected patients signed informed consent and were examined at routine endoscopy (GIF-260 Olympus Optical Co, Tokyo, Japan) and then the patients with no exhibiting esophageal mucosal injury at routine endoscopy were selected for staining. The steps of staining were as follows: For the selected patients, the spray catheter of endoscope was ported into for biopsy and all mucus rinse in the esophageal mucosal surface was washed by 50 ml distilled water. Subsequently, a total of 20 ml 1.5% Lugol's iodine solution was sprayed on the esophageal mucosa from top to down uniformly, stay for 30-60 seconds, and was washed by distilled water. Finally, mucosal staining was observed and photographed, and then all residual iodine solution in the stomach and esophagus was cleaned from patients by aspirator. All examinations were performed by two experienced doctors.

Staining Analytical Standards

Normal staining: Lugol's iodine solution produced a uniform staining between the lesions with the surrounding tissues. The shallow staining: Lugol's iodine solution produced a shallower staining in the lesions than the surrounding tissues and our naked eye can distinguish the clear boundaries well. Non-staining: the lesion was not stained, clear boundaries with the surrounding tissues. Excessive staining: Lugol's iodine solution produced a dark brown staining than surrounding tissues and the dark was significantly than the surrounding tissue and even to be stained sepia. Images were assessed and determined by two experienced doctors.

Gerd Q Scoring Criteria

Gerd Q was made by Dent according to Diamond's research[9], Scores of Gerd Q depended on the frequency of disease symptoms in the past seven days (0 score, never or 1 score, once or 2 score, 2 to 3 times or 3 score, 4 to 7 times). Symptoms including two positive symptoms (heartburn and regurgitation), two negative symptoms (abdominal pain and nausea) and two symptoms affecting the quality of life (sleep disorders due to heartburn/regurgitation and require additional antacids due to heartburn/regurgitation). Gerd Q scoring was based on the onset and frequency integrator about the three kinds of symptom (Table I).

Tissue Biopsy

Tissue samples were stained by HE (hematoxylin-eosin) method, and with 10 × 20 magnification. The patients with no exhibiting mucosal injury at routine endoscopy were stained by Lugol's iodine solution, and the patients with uniform staining were bypassed biopsy, but 2-3

Table I. Gerd Q score.

Item	The symptoms in past seven days	0 d	1 d	2-3 d	4-7 d
Positive symptoms	Heartburn	0	1	2	3
	Regurgitation	0	1	2	3
Negative symptoms	Abdominal pain	3	2	1	0
	Nausea	3	2	1	0
Positive influence	Sleep disorders	0	1	2	3
	Additional medication	0	1	2	3

biopsies were obtained from the esophageal mucosa area with shallow staining and/or non-staining for histological examination.

Medical Treatment

There were 32 selected patients were treated with esomeprazole (trade name: Nexium, AstraZeneca, Specification: 20 mg/tablet) 20 mg twice a day and mosapride citrate (trade name: Gasmotin, Sumitomo Pharmaceuticals (Suzhou) Co., Ltd., Specification: 5 mg/tablet) 5 mg 3 times a day at 30 min before the meal for 4 weeks. Other antacids and antispasmodic agents that have influence on gastrointestinal motility were interrupted during the treatment. Before and after treatment, all of the changes of symptoms (such as heartburn and acid regurgitation) and Gerd Q score were recorded. Meanwhile, the difference about the staining in esophageal mucosa of NERD patients and histological changes were also recorded.

Statistical Analyses

The SPSS13.0 soft was used to statistical analysis (SPSS Inc., Chicago, IL, USA). All measurement data was expressed as mean ± standard deviation (X ± S) and the count data of two groups was analyzed by χ^2 test.

Results

Clinical Data

A total of 96 GERD patients were selected, including 68 NERD patients and 28 EE patients. The proportion of women in the NERD patients group was obvious higher than the EE group (male/female: 34/34 and 20/8, $p < 0.05$). The average age of NERD group was obvious smaller than the EE group [(42.32 ± 3.99) years and (49.00 ± 7.18) years, $p < 0.05$]. The demographic characteristics such as BMI, dietary preferences, smoking, drinking and others had no statistically significant difference ($p > 0.05$) in these two groups. Meanwhile, there was no statistically significant difference between these two groups of patients in the course of the disease ($p > 0.05$) (Table II).

Staining Results Analyses

For the 68 NERD patients, there were 36 patients in the uniform staining group and 32 patients in the shallow staining and/or non-staining group. The gender composition (male/female: 20/16 and 17/15, $p > 0.05$), the age distribution (41.58 ±4.94 years and 43.16 ± 2.36 years, $p > 0.05$) and the course of the disease ($p > 0.05$) were not significant difference ($p > 0.05$) in these two groups.

Lugol's iodine staining was carried out on the 68 NERD patients. For the esophageal mucosa with uniform staining ones (36 patients) (see Figure 2) were not take a biopsy, but for the esophageal mucosa with shallow staining and/or non-staining (32 patients) (see Figure 3), 2 ~ 3 biopsies were obtained for histological examination. The 32 patients with shallow staining and/or non-staining were treated for 4 weeks and stained by Lugol's iodine solution. We observed 28 patients with uniform staining and 4 patients with shallow staining and/or non-staining at endoscope, then 2-3 biopsies were gained in each of the 4 patients for histological examination.

Table II. The general condition of patients in NERD group and EE group.

	Man/woman	Average age	BMI	Smoke/no smoking	Drink/no drinking
NERD	34/34	42.32 ± 3.99	23.72 ± 1.77	27/41	29/39
EE	20/8	49.00 ± 7.18	24.13 ± 2.01	16/12	17/11
p	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05

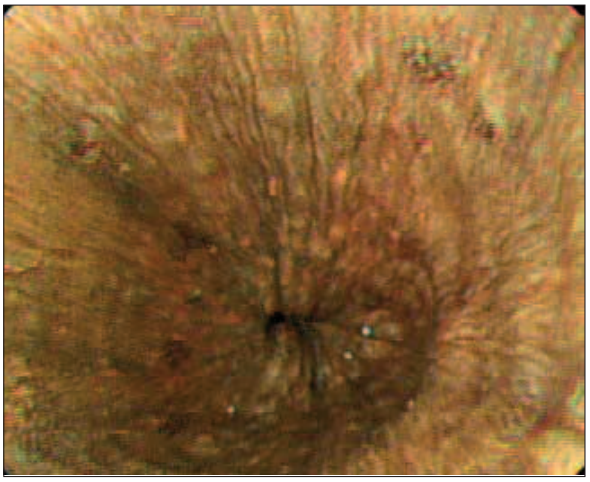

Figure 2. The low esophageal mucosa stained by Lugol's iodine solution showed uniform.

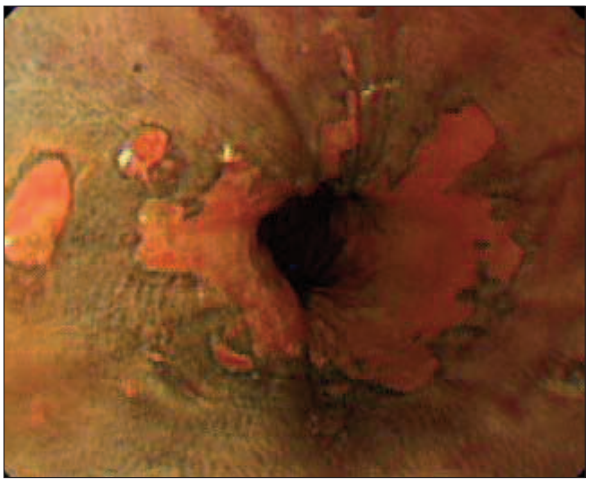

Figure 3. The low esophageal mucosa stained by Lugol's iodine solution showed some shallow staining and/or non-staining in some pieces.

Gerd Q score Analysis

Only the 68 NERD patients were rated for Gerd Q score. Before the treatment, Gerd Q score in the uniform staining group was 9.78 ± 2.56 and in the shallow staining and/or non-staining group was 10.26 ± 2.12. After 4 weeks for treatment, Gerd Q score in the uniform staining group was 6.98 ± 2.02 and in the shallow staining and/or non-staining group was 6.82 ± 1.96. Before the treatment, there was no statistically significant difference ($p > 0.05$) between the uniform staining group and the shallow staining and/or non-staining group in Gerd Q score. After the treatment, there was also no statistically significant difference ($p > 0.05$) between the uniform staining group and the shallow staining and/or non-staining group in Gerd Q score. However, there was statistically significant difference ($p < 0.05$) between the uniform staining groups which were treated before and after in Gerd Q score, meanwhile, there was statistically significant difference ($p < 0.05$) between the shallow staining and/or non-staining groups which were treated before and after in Gerd Q score (Table III).

Histomorphology Analysis

Before the treatment, there were 32 NERD patients with shallow staining and/or non-staining and the histomorphology as follow: 28 patients with squamous cell hyperplasia and 4 patients with papillomatosis. After 4 weeks for treatment, 28 patients showed uniform staining (without histological examination), 4 patients showed shallow staining and/or non-staining and the histological features of these regions appeared as follow: three with squamous cell hyperplasia and one with papillomatosis.

Discussion

NERD is the most common type of GERD and accounting for about 70% of GERD[10], also known as endoscopic negative reflux disease (ENRD), symptomatic gastroesophageal reflux disease (symptomatic GERD). This study showed that the NERD patients accounting for 70.8% of the total number of GERD patients (68/96), which was consistent with the results of

Table III. Comparison of Gerd Q socre.

NERD group	Number	Prior-treatment Gerd Q score	Post-treatment	*p*
Esophageal uniform staining group	36	9.78 ± 2.56	6.98 ± 2.02	< 0.05
Esophageal shallow staining and /or non-stain group	32	10.26 ± 2.12	6.82 ± 1.96	< 0.05
p		> 0.05	> 0.05	

previous studies[10]. Recently, NERD was reported as a relatively independent disease in GERD and which has its own particular pathogenesis, diagnosis and treatment. However, there was no uniform diagnostic criterion for NERD[1,11]. In this study, we explored the clinical characteristics of the NEDR by Lugol's iodine solution staining at routine endoscopy and combined with Gerd Q score and histological examination.

Lugol's iodine solution is a kind of compound iodine solution and belongs to the chromoendoscopy. The mechanism of staining is that Lugol's iodine solution produces a uniform greenish-brown staining of the normal mature non-keratotic squamous epithelium, because glycogen present in the normal mature non-keratotic squamous epithelium interacts with the iodine in Lugol's solution. The esophagitis, especially for associated with squamous cell hyperplasia, having little glycogen remained and is unstained[12-13]. Lugol's iodine solution was widely applied in the examination by routine endoscopy for the early diagnosis of esophageal cancer and its intraepithelial neoplasia[14-15]. However, the report about the application of Lugol's iodine solution in NERD was relatively rare. Makoto et al[16] used Lugol's iodine solution found the basal cells of esophageal mucosa thickening, nipple extending and lymphocytic infiltrateding in NERD patients, which showed the significant difference between unevenly staining and uniform staining. Hoffman et al[17] applied I-Scan high definition endoscopy with Lugol's iodine solution staining to improve the detection rate of mucosal injury in NERD. However, the study about the routine endoscopy combined with Lugol's iodine solution staining in NERD was rare. In this study, before the treatment, the routine endoscopy combined with Lugol's iodine solution showed that the population of uniform staining and the shallow staining and/or non-staining in NERD patients was 52.9% (36/68) and 47.1% (32/68), respectively. There were 32 patients who were treated for 4 weeks and stained by Lugol's iodine again, and then we found 28 of 32 patients with shallow staining and/or non-staining became uniform staining at endoscopy and the population of uniform staining was 87.5%. The results indicated that the esophageal squamous epithelial hyperplasia had decreased and the esophageal mucosa glycogen was increased for the NERD patients who were treated for 4 weeks. Therefore, the routine endoscopy combined with Lugol's iodine staining can be used to diagnose NERD and serve as a detected method to estimate the treatment effect in the primary hospitals that lack advanced endoscope imaging techniques.

According to the GERD patients with specific symptoms, Montreal consensus suggested to diagnose GERD depended on these symptoms[18]. Accordingly, scholars had developed a quantitative assessment questionnaire diagnostic tool[18]. Gerd Q was the most representative one and its effectiveness was verified by Dent and other the internationally renowned digest experts[10,19]. Community Medical Center in western countries surveyed for 300 GERD patients and the results believed the Gerd Q with sensitivity (65%) and specificity (71%) for confirmed GERD patients[4]. Jones et al believed Gerd Q with the accuracy of diagnosis for GERD, and can be used to assess the impact of the disease on quality of life and treatment effects[20]. Della Casa et al[21] considered that Gerd Q can avoid the complicated checks in clinical application and reduce the patient's pain, cost and waste of medical resources. However, the report about that Gerd Q was used to evaluate NERD was rare. We believed the Gerd Q score is simple, fast, sensitivity, specificity and can be used to access the symptoms in NERD patients and to evaluate the treatment effect. In this study, before treatment, there was no statistically significant difference among reflux esophagitis group (11.46 ± 2.42) and the uniform staining group (9.78 ± 2.56) and the shallow staining and/or non-staining (10.26 ± 2.12) in Gerd Q score. After treated for 4 weeks, there was also no statistically significant difference ($p > 0.05$) in the Gerd Q score between uniform staining group and shallow staining and/or non-staining group. Before and after treatment, there was statistically significant difference ($p < 0.05$) in the Gerd Q both uniform staining groups and shallow staining and/or non-staining groups. However, there was no statistically significant difference ($p > 0.05$) in the Gerd Q score between the prior-treatment group and the post-treatment group. These results implied the symptoms of NERD were relieved after treatment. Meanwhile, the esomeprazole combined with mosapride citrate was effective to cure NERD.

Tobey et al[22] found non-erosive acid damage of esophageal epithelial cells with some features such as cell gap widened, resistance decreased, permeability increased, and they believed these features explain the reasons for non-erosive esophagitis proliferation of basal cells. In addition, studies had shown that the use of esomeprazole for treatment made the cell gap widened sig-

nificantly restored in patients[23]. Meanwhile, to observe the changes in morphology and diameter of esophageal mucosa intraepithelial papillary capillary loop, the confocal laser endomicroscopy was used as a new model tool for real-time simulation of pathological diagnosis, and the cell gap cut-off was first identified as 17.2 m in NERD patients[24]. In this study, before the treatment, there were 32 patients with shallow staining and/or non-staining. Furthermore, there were 28 of 32 patients with squamous cell hyperplasia and 4 of 32 patients with papillomatous hyperplasia. However, after 4 weeks for treatment, 28 of 32 patients with shallow staining and/or non-staining became uniform staining at endoscope and 4 of 32 patients still shown shallow staining and/or not staining. Furthermore, we found 3 of 4 patients with squamous cell hyperplasia and 1 of 4 patients with papillomatosis hyperplasia. These results indicated that the cell gap and squamous epithelial hyperplasia in esophageal epithelial cells both were reduced after 4 weeks of treatment and also indicated our study was consistent with previous studies[23-24].

Because of most of biopsy specimens were taken from esophageal surface, this study only found the changes of squamous epithelium hyperplasia and papillomatosis in NERD patients. More meticulous observations were not carried out. Before and after treatment, the patients with uniform staining or shallow staining and/or non-staining were not classified in the squamous epithelial hyperplasia, the inflammatory cell infiltration, the cell gap extended and the papillomatosis hyperplasia. In addition, in this paper, the consistent analysis of inter-observer and intra-observer was lack result from fewer samples. More meticulous researches and more profound understanding of NERD will be carried out in the future.

Conclusions

Lugol's iodine solution staining combined with endoscope, Gerd scoring and histomorphology could be used to evaluate the diagnosis and therapeutic effect of NERD. Our studies provide an effective, simple and low cost method to diagnose and evaluate therapeutic effect of NERD. However, more studies are needed to confirm our methods.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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