European Review for Medical and Pharmacological Sciences 2016; 20: 476-485

Anti-reflux anastomosis following esophagectomy for adenocarcinoma of the esophagogastric junction: impact of duodenogastroesophageal reflux and expression of cyclooxygenase-2 in the remnant esophagus

J.-D. WANG[1], S.-Y. ZHU[1], Y.-J. LU[1], L.-Y. GAO[2]

[1]Department of Thoracic Surgery, First People's Hospital in XuZhou City, XuZhou, China.
[2]Department of Pathology, First People's Hospital in XuZhou City, XuZhou, China.

Abstract. – OBJECTIVE: Reflux is the principal complication for patients after esophagectomy with gastric reconstruction. The aim of this study was to investigate the effect of the modified Nissen fundoplication after resection of adenocarcinoma from the esophagogastric junction (AEG) on controlling the reflux and the role of duodenogastroesophageal reflux (DGER) and cyclooxygenase-2 (COX-2) expression level in the remnant esophagus.

PATIENTS AND METHODS: Sixty patients with AEG were randomly divided into two groups: (i) the conventional anastomosis group and (ii) the anti-reflux anastomosis group. Fifty esophagectomized patients were invited to participate in postoperative follow-up after 6 to 12 months. Among those we had 29 cases in the conventional anastomosis group and 21 in the anti-reflux anastomosis group. We used endoscopy, simultaneous 24 hours esophageal pH and bilirubin monitoring in this study. The COX-2 expression level in the remnant esophagus was detected using real-time PCR.

RESULTS: The reflux esophagitis prevalence in anti-reflux anastomosis group was comparable to that in the conventional group ($p = 0.154$). De-Meester score and fraction time of bilirubin abs >0.14 decreased more intensely in the anti-reflux anastomosis group ($p < 0.05$). The COX-2 expression level in of anti-reflux anastomosis group was evidently lower than that in the conventional anastomosis group ($p = 0.022$) while it was meaningfully higher compared to the normal control group ($p = 0.046$). COX-2 up-regulation as well as high prevalence of esophagitis were observed in simultaneous acid reflux and DGER ($p < 0.05$).

CONCLUSIONS: Although modified fundoplication following resection of AEG did not achieve an optimal effect on controlling reflux, it was very effective in decreasing the reflux. COX-2 expression monitoring can be considered as a possible new way to evaluate the impact of anti-reflux surgery. DGER occurring in acidic environment could develop severe reflux esophagitis and up-regulate the COX-2 expression.

Key words:
Nissen fundoplication, Acid reflux, DGER, AEG, COX-2.

Introduction

Partial esophagectomy and proximal gastrectomy are major surgical procedures for patients suffering from adenocarcinoma of esophagogastric junction (AEG). In these cases, we have less than 20%, five years survival rate[1]. Gastric and duodenal reflux after esophagectomy with gastric conduit reconstruction is a common problem and is often considered an inevitable consequence of this type of surgery. Reflux symptoms have been found in 60 to 80% of esophagectomy cases[2] and problems such as severe heartburn and regurgitation associated with this condition can create physical discomfort as well as social complications. Presently, the life quality after a surgical intervention has become paramount. There are gathering evidences indicating that gastroesophageal reflux almost occurs in all patients who underwent esophagectomy and in nearly all cases the conventional anastomotic orifice fails to prevent gastroesophageal reflux after the surgery[3].

The prevalence of esophagitis and Barrett's esophagus in the esophageal stump is respectively 45.9 to 91.9% and 29.5 to 57.5% after the surgery. The development of Barrett's esophagus might result in a new carcinoma in patients who have achieved long-term survival after esophagectomy[4].

Modified Nissen fundoplication shaped by the gastric fundus wrapping around the remnant oesophagus and conventional anastomosis after esophagectomy may be considered as a powerful anti-reflux manoeuvre in patients with esophageal

Corresponding Author: Wang JD, MD; e-mail: wangjd165@163.com

carcinoma[5]. Its anti-reflux effect was demonstrated by endoscopy and 24-hour pH monitoring[6]. Modified Nissen fundoplication is shaped by an adequate width and length of remnant gastric tube wrapping around the esophageal stump and anastomosis. However, the effectiveness of this improved surgical procedure in controlling gastroesophageal reflux is still unclear. To investigate the anti-reflux effect of this method and the impact of DGER on reflux esophagitis we used endoscopy, simultaneous ambulatory 24 hours pH and spectrometric bilirubin monitoring following the AEG resection.

Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins pathway. COX-2 is involved in the regulation of a broad range of cellular processes including proliferation, angiogenesis and resistance to apoptosis. According to the prior studies in this field, among many genes evaluated in the distal esophageal squamous mucosa, only COX-2 expression well correlated with the amount of esophageal acid exposure[7]. The increased expression levels seen in some genes, such as COX-2 and interleukin 8 (IL-8), in the distal esophageal mucosa, were significantly lowered and returned to the level comparable to that seen in the squamous mucosa of patients without reflux disease[8,9]. Based on these findings, using real-time PCR, the COX-2 expression level in the remnant esophagus was measured in order to verify whether our anti-reflux surgery can change COX-2 expression in the remnant esophagus. We also evaluated the effects of acid reflux and DGER on COX-2 expression.

Patients and methods

Patients

From December 2011 to July 2013, sixty patients with adenocarcinoma of esophagogastric junction (AEG) were chosen and randomly divided into two groups: (i) the conventional anastomosis group (modified Nissen fundoplication added to conventional anastomosis) and (ii) the anti-reflux anastomosis group. We had 39 males and 21 females, with ages ranging from 40 to 76 years (mean age was 60.2 years). The pro-operative general condition, the size of the tumor and pathologic type were analogical between the two groups. Fifty post-operative patients including 37 males and 13 females, aging from 40 to 73 years (average age 61.6 years) took part in the follow-up studies (6 to 12 months after operation). This included 29 patients in the conventional anastomosis group and 21 patients in the anti-reflux anastomosis group. All postoperative patients received endoscopy examination while thirty- seven patients underwent 24-hours pH and bilirubin monitoring. The COX-2 expression level in the remnant esophagus was evaluated using real-time PCR.

Demographic and clinic pathological information such as age, gender, characteristics of AEG and the method of surgery were obtained from patient's records (Table I). The pathologic stage of AEG was determined according to the 7th edition of the esophageal adenocarcinoma and gastric cancer TNM classification of the American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC). According to UICC, a tumor with an epicenter located within 5 cm from the EGJ with extension into EGJ and the distal esophagus (AEG type I and II) was staged according to the esophageal adenocarcinoma staging scheme. Tumors with an epicenter greater than 5 cm from EGJ or those within 5 cm of the EGJ without extension into EGJ (AEG type III) were staged using the gastric carcinoma staging scheme[10]. Twelve healthy volunteers with no reflux and reflux esophagitis were selected as normal control for this study. These included ten males and two females with mean age of 45.6 years. They were selected using 24-hour esophageal pH and bilirubin monitoring and endoscopy. Written informed consent was obtained from postoperative patients and volunteers. This study was approved by Hospital Ethics Committee.

Surgical procedure

All patients underwent surgery via left thoracotomy and the esophagus and stomach were mobilized. The esophagus was mobilized to the level of inferior lung vein. The partial distal esophagus, gastroesophageal junction, and proximal stomach were resected at 5 cm tumor-free margin. Remaining parts of the stomach were shaped into gastric tube 3 to 4 cm in width using a linear stapling device (GIA6038S, Covidien, Saint Louis, MO, USA). The anastomosis was performed below the aortic arch. The conventional anastomosis was at the terminal side of the esophagogastric anastomosis using a circular stapling device (HuaSen 24#, ChangZhou Medical Equipment Company, ChangZhou, China) and was shaped on the apex of the gastric tube (Fig-

Table I. Clinicopathologic data in patients with adenocarcinoma who underwent partial esophagectomy and proximal subtotal gastrectomy with the conventional anastomosis and the anti-reflux anastomosis.

Clinicopathologic characteristics	Conventional anastomosis	Anti-reflux anastomosis	Total
Gender (n)			
Male	21	16	37
Female	8	5	13
Age (mean year)	62.3	58.5	
Siewert and Stein classification (n)			
Type I	4	3	7
Type II	18	15	33
Type III	7	3	10
Turmor size (cm)	1.5-5	1-4	
Histology (n)			
Papillary adenocarcinoma	12	7	19
Tubular adenocarcinoma	7	9	16
Mucinous adenocarcinoma	4	2	6
Poorly differentiated adenocarcinoma	5	3	8
Other types	1	0	1
Tumor stage (n)			
Ia	0 (0)	0 (0)	0
IIb	0 (0)	1 (0)	1
IIa	3 (1)	4 (1)	9
IIb	7 (3)	7 (1)	18
IIIa	9 (2)	5 (1)	17
IIIb	2 (1)	1 (0)	4
IIIc	1 (0)	0 (0)	1
IV	0 (0)	0 (0)	0

The data in bracket was staged according to the gastric carcinoma staging scheme.

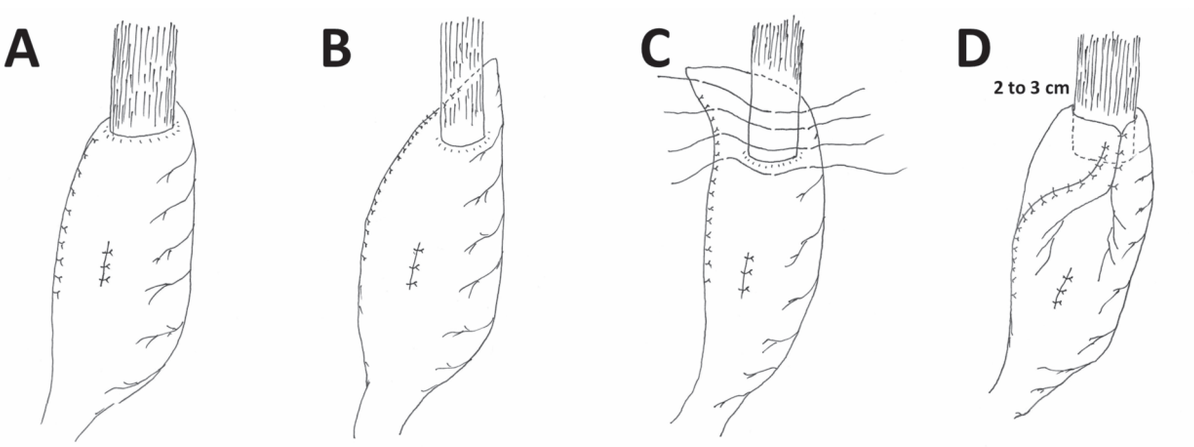

Figure 1. Schematic diagram of conventional anastomosis **(A)** and anti-reflux anastomosis (**B** to **D**). **A,** The conventional esophagogastric anastomosis was the end to side esophagogastric anastomosis using a circular stapling device in apex of gastric tube and near to greater curvature of gastric tube. **B,** The esophagogastric anastomosis was performed in anterior wall of the stomach, which was in position 3 to 4 cm below the apex of gastric tube and near to greater curvature of the gastric tube. **C,** The gastric tube apex was drawn to left at the remnant esophagus. Four non-absorbable sutures were sewn from the gastric wall at side of the greater curvature of stomach to the gastric wall at side of lesser curvature of stomach. The upper two sutures included the terminal remnant esophagus wall. The third suture was only sewn at gastric wall between side of the greater curvature of stomach and lesser curvature of stomach. Fourth suture included anterior wall of the stomach below 1 cm anastomosis. **D,** The three sutures were tied tight to complete the modified Nissen fundoplication following the conventional esophagogastric anastomosis.

ure 1A). For the modified Nissen fundoplication (Figure 1B-D), the anastomosis was shaped on the anterior wall of the stomach, which was in a position 3 to 4 cm below the apex of the gastric tube and near to the greater curvature of the gastric tube. After conventional anastomosis, the apex of the gastric tube was drawn to the left side of the remnant esophagus. This was placed in a way that the gastric tube extended beyond the two sides of the remnant esophagus. The fundoplication was sewn with four non-absorbable sutures. The four sutures, which were sewn to gastric wall at the greater curvature side of gastric tube to gastric wall at lesser curvature of gastric tube side, passed across the terminal remnant esophagus and anastomosis. The first, second and fourth sutures were sewn at the wall of terminal remnant esophagus and anterior wall of the stomach 1cm below anastomosis. The third suture was only sewn at the gastric wall from the greater curvature to the lesser curvature of the gastric tube. All the sutures were placed 1 cm apart to close the wrap. The fundoplication was performed by drawing the lesser curvature of stomach's gastric tube side forward and right to the terminal remnant esophagus and anastomosis, wrapping it around the terminal esophageal remnant 2 to 3 cm and the anastomosis and securing it to the gastric wall at side of the greater curvature of the stomach. The pyloroplasty was not performed in the surgical procedure.

Endoscopy

Six months after the surgery, the remnant esophagus mucosa was examined using a flexible endoscope (Olympus GIF-Q260J; Olympus Optical Co., Ltd., Tokyo, Japan). The severity of reflux esophagitis observed during the endoscopy was graded according to Los Angeles (LA) classification system[11]. Three biopsy specimens were collected at 3, 9, 12 clock position of the remnant esophageal mucosa 3 cm above of esophagealgastric anastomosis in postoperative patients and in the same region of distal esophageal mucosa 3 cm above esophagogastric junction (Z-line) in healthy volunteers.

Ambulatory 24 hours esophageal pH and bilirubin monitoring

Simultaneous esophageal pH and bilirubin monitoring were performed for 24 hours. Antimony with single sensor for pH (Medtronic, Skovlunde, Denmark) was calibrated in buffer solutions of pH 1.07 and pH 7.01 before each study. Then, the fiber optic probe for bilirubin monitoring (Medtronic) was calibrated with water. After the catheters had been passed trans nasally, pH sensor and bilirubin monitoring were positioned in the region of remnant esophagus 5 cm above anastomotic orifice identified fluoroscopically by the presence of the staple in esophagectomized patients and in the region of distal esophagus 5 cm above esophagogastric junction (identified by endoscopy) in healthy volunteers. The esophageal pH and bilirubin monitoring was respectively recorded by a portable digital data recorder (Digitrapper Mark III, Medtronic) and a portable optoelectronic data recorder (Bilitec 2000, Medtronic). Data were downloaded into a personal computer for further analyses using Esophogram Reflux Analysis Software (Medtronic). Elevated acid reflux was defined as DeMeester score above 14.72[12] and elevated duodenal gastro esophageal reflux (DGER), was defined as bilirubin absorbance exceeding 0.14 for more than 1.8% of the monitoring time[13].

Real-time PCR

Histological biopsy specimens underwent routine fixation. Total RNA from the biopsy samples was extracted using High Pure FFPET RNA Isolation Kit according to the instructions provided by the supplier (Roche Applied Science Co., Basel, Switzerland). Total RNA samples (1 μg) were subjected to reverse transcription in order to obtain complementary DNA (cDNA). The reaction was performed in 25 μl according to Transcriptor first strand cDNA synthesis kit (Roche Applied Science Co.). Real-time PCR measurement of COX-2 cDNA was performed on an ABI PRISM 7500 sequence detector (PE Applied Biosystems, Foster City, CA, USA) with TaqMan assay. The COX-2 primers and probe sequences were synthesized (PE Applied Biosystems) as described previously[14] (Table II). The PCR reaction mixture consisted of 1200 nmol/of each primer, 200 nmol/probe, 0.4 U of AmpliTaq Gold Polymerase, 200 nmol/each dATP, dCTP, dGTP, dTTP, 3.5 mM MgCl2 and 1x Taqman Buffer A containing a reference dye, to a final volume of 20 μl (all reagents from PE Applied Biosystems). Cycles conditions were 50°C for 2 min, 95°C for 10 min, followed by 46 cycles at 95 °C for 15s and 60 °C for 1 min. TaqMan measurements yield Ct values were inversely proportional to the amount of cDNA in the tube. A higher Ct value indicated that more PCR cycles were required to

Table II. Primers and Probes of COX-2 and β-actin.

GenBank accession: NM-000962
Forward primer of COX-2 5′-GCTCAAACATGAT-GTTTG CATTC-3′
Reverse primer of COX-2 5′-GCTGGCCCTCGCTTATGA-3′
TaqMan probe of COX-2 6FAM 5′-TGCCCAGCACTTCACGCATCAGTT-3′ TAMRA.
GenBank accession: NM-001101
Forward primer of β-actin 5’-GAGCGCGGCTACAGCTT-3’
Reverse primer of β-actin 5’-TCCTTAATGTCACGCACGATTT-3’
TaqMan probe of β-actin 6FAM 5′-ACCACCACGGCCGAGCGG-3′TAMRA.

reach the level of detection. Gene expression values (relative mRNA levels) were communicated as ratios (differences between the Ct values) of COX-2/β-actin in arbitrary units. An internal reference gene (β-actin) provided a normalization factor for the amount of RNA isolated from a specimen.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (Version 19.0; SPSS, Inc., Chicago, IL, USA). The prevalence of reflux esophagitis and gastroesophageal reflux were analyzed by Chi Square test. The 24 hours pH and bilirubin monitoring parameters were compared by Wilcoxon rank-sum test. The COX-2 expression was estimated using Scheffe test. A p-value of ≤ 0.05 was considered statistically significant.

Results

Using endoscopy examination, different grades of reflux esophagitis were observed in the conventional anastomosis as well as the anti-reflux anastomosis groups (Figure 2). The reflux esophagitis occurrences were respectively 69% and 47.7% in the conventional anastomosis and the anti-reflux anastomosis groups. Although reflux esophagitis prevalences in the anti-reflux anastomosis group declined more intensely, differences between the groups were not statistically significant (χ^2=2.313, p=0.154).

The reflux patterns were classified into four types: (i) neither acid reflux nor DGER, (ii) only acid reflux, (iii) only DGER and (iv) simultaneous acid reflux and DGER by analysis of 24-hour

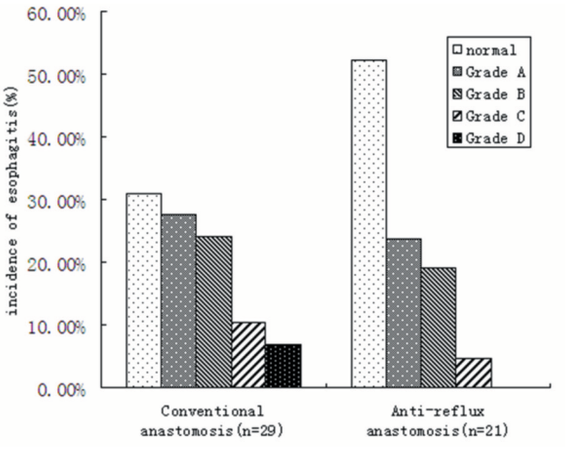

Figure 2. Prevalence of different grade of reflux esophagitis between the groups of conventional anastomosis and anti-reflux anastomosis. There was no difference in prevalence of reflux esophagitis between two groups (χ^2=2.313, p=0.154).

pH and bilirubin monitoring (Figure 3). The reflux occurrences were respectively 85.2% and 76.5% in the conventional anastomosis and anti-reflux anastomosis groups. No significant difference in reflux occurrence was observed between the two groups (χ^2=0.343, p=0.434). The simultaneous acid reflux and DGER were the main reflux patterns in both groups. Higher reflux esophagitis occurrences (grade C and grade D) were observed patients with simultaneous acid reflux and DGER (χ^2=4.723, p=0.043) (Figure 4). DeMeester score and fraction time of bilirubin abs >0.14 in the anti-reflux anastomosis

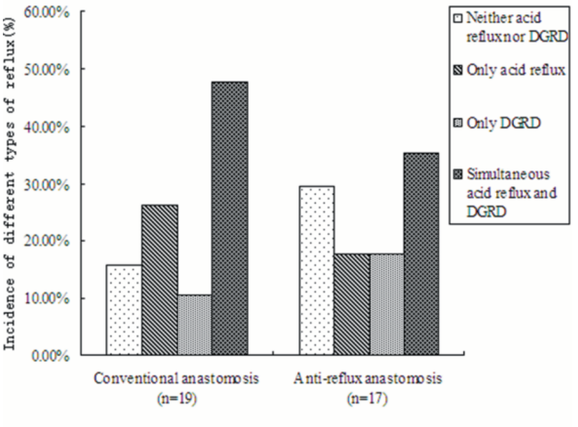

Figure 3. Incidence of different types of reflux between the groups of conventional anastomosis and anti-reflux anastomosis. No difference was observed in prevalence of reflux between two groups (χ^2=0.343, p=0.434).

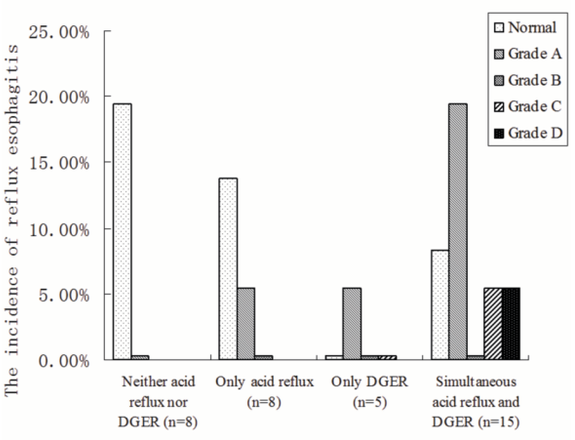

Figure 4. Prevalence of various extents of reflux esophagitis in different reflux patterns. The higher occurrence of reflux esophagitis of grade C and grade D was observed in the patients who had simultaneous acid reflux and DGER compared with that in only DGER and only acid reflux (χ^2=4.723, p=0.043).

group were clearly dropped more than those in the conventional anastomosis group and differences were statistically significant (p<0.05) (Table III).

COX-2 expression level was lowest in the normal control group (0.053±0.037). In patients underwent esophagectomy, the COX-2 gene expression level was 0.126±0.085 for patients with normal esophageal mucosa (n=20) and 0.147±0.112 in patients with reflux esophagitis (n=30). The COX-2 expression levels in esophagectomized patients with normal esophageal mucosa and reflux esophagitis were clearly higher compared to that in the normal control (p=0.0314, 0.012) and there was no significant difference between esophagectomized patients with normal esophageal mucosa and reflux esophagitis (p=0.1281). The COX-2 expression levels in patients with simultaneous acid reflux and DGER (0.2301±0.0571) were evidently higher than that in only acid reflux (0.0901±0.0546, p=0.004,) and control group (p=0.001). However, the difference was not significant when compared to that in DGER only (p=0.142) (Figure 5).

COX-2 expression levels in the remnant esophagus mucosa are shown in Figure 6. The COX-2 expression level in anti-reflux anastomosis group was evidently lower compared to that in the conventional anastomosis group (0.192±0.148 *vs.* 0.114±0.087, p=0.022), while it was significantly higher when compared to the normal control group (0.114±0.087 *vs.* 0.053±0.037, p=0.046).

Discussion

Adenocarcinoma of the esophagogastric junction (AEG), which occurs within 5 cm of the esophagogastric junction (EGJ), can be divided into three types according to Siewert and Stein classification of AEG[15]. There are several possible approaches for surgical resection of carcinoma of the esophagogastric junction. These included resection of the proximal stomach and total gastrectomy, and proximal gastrectomy with partial esophagectomy being the main surgical procedure for AEG. AEG patients who underwent partial esophagectomy and proximal gastrectomy with gastric tube reconstruction have a high risk of prolonged esophageal exposure to gastric acid and duodenal juice because the normal anti-reflux mechanisms (lower esophageal sphincter, angle of His, diaphragmatic sling) have been resected. Zang et al[16] reported that 60% of patients after surgery for AGE (cardiac cancer) had typical symptoms of gastroesophageal reflux and all patients with AGE suffered from postoperative gastroesophageal reflux by 24-hour pH monitoring. Manual anastomosis, such as encasing-in style and "scarf" style, could not decrease gastroesophageal reflux. The height at which the anastomosis is shaped is considered to

Table III. Comparison of acid reflux and DGER between conventional and anti-reflux anastomosis groups.

Group	Conventional Anastomosis (n=19)	Anti-reflux Anastomosis (n=17)	*H*	*p*
	x±SD	x±SD		
DeMeester score	132.72±83.01	56.18±48.18	2.758	0.0061
Fraction time of bilirubin abs>0.14	17.28±13.15	7.62±8.37	2.521	0.0118

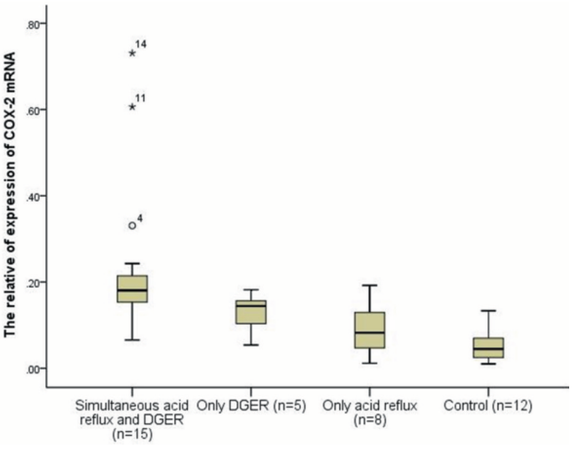

Figure 5. COX-2 expression in the remnant esophageal mucosa in acid reflux and DGER profile in 36 patients including the conventional anastomosis and anti-reflux anastomosis groups. Boxes represent the range of standard deviation with the horizontal line representing mean value. Error bars represent maximum and minimum values. The COX-2 expression in simultaneous acid and DGER was evidently higher than that in only acid reflux and control (p=0.004, p=0.001). Difference was not significant when compared to that in only DGER (p=0.142).

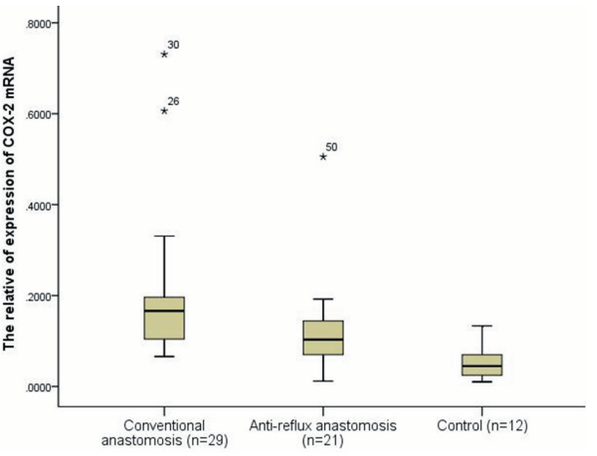

Figure 6. COX-2 expression in the remnant esophageal mucosa among the conventional anastomosis, anti-reflux anastomosis and control groups. Boxes represent the range of standard deviation with the horizontal line representing mean value. Error bars represent maximum and minimum values. The COX-2 expression in the anti-reflux anastomosis group was evidently lower than that in the conventional anastomosis group (p=0.022). COX-2 expression in the conventional anastomosis group significantly increased compared to the normal control group (p=0.046).

affect the extent of the reflux. More severe refluxes occur on the lower anastomosis locations[17,18] because in lower anastomosis cases, a bigger part of stomach is subjected to positive intra-abdominal pressure thus promoting greater reflux[19]. Since conventional anastomosis fails to prevent gastroesophageal reflux, many surgical techniques attempting to control reflux after esophagectomy have been developed[20-24]. However, these surgical procedures are too complex and very challenging and are unsuitable for radical resection of esophageal cancer and AEG. Hence, these improved surgical procedures have not been commonly applied as standard techniques.

Treatment of patients with gastroesophageal reflux disease by Nissen fundoplication is a well-established surgical technique. Aly et al[5] originally reported that the modified Nissen fundoplication was added to conventional anastomosis in esophagectomized patients with esophageal cancer and postoperative reflux symptoms, were reduced in the majority of patients with this anti-reflux maneuver. Our previous study also demonstrated that this technique was effective in controlling reflux[6]. In AEG patients when proximal stomach must be resected, sufficient width and length of remnant stomach is very important because it should wrap around the terminal part of remnant esophagus and anastomotic orifice in order to satisfy Nissen fundoplication. In our case series, the width of remnant gastric tube was more than 4 cm, and that the gastric tube apex overtopped 3 cm of anastomotic site on the anterior wall of the remnant stomach. In addition, the tumor, which was less than 4 cm in diameter, located in EGJ extension to distal esophagus and less gastric curvature (AEG type I and II) or in the gastric fundus without extension to GEJ and gastric body (AEG type III). Hence, the location and the size of tumor were important factors to successfully complete the modified Nissen fundoplication following resection of AEG.

Our data showed that various patterns of reflux occurred in both groups and the simultaneous acid reflux and DGER was the main reflux pattern. Although reflux pattern was comprised of three types: only acid reflux, only DGER and simultaneous acid reflux and DGER, it was possible for patients with only DGER to have acid reflux due to the alkaline content in DGER neutralizing gastric acid. Previous findings demonstrated that most esophageal bilirubin exposures occurred in a pH ranging from 4 to 7 in esophagectomized patients[25]. Marshal et al[26] described that esophageal mucosal injury was minimal in patients with isolated bile reflux. Vaezi et al[27] argued that duodenal contents required an acid environment to produce more severe mucos-

al injury. Our findings showed that severe esophagitis (grade C and D reflux esophagitis) occurred only in DGER as well as simultaneous acid and DGER, rather than in acid reflux only. Furthermore, we observed higher occurrence of severe esophagitis in simultaneous acid and DGER. This suggested that DGER played a significant role in damaging the esophageal mucosa and the injury might be more severe when DGER was exposed to acidic environment.

Combined ambulatory esophageal 24 hours pH and bilirubin monitoring could detect changes in acid reflux and DGER. Our results showed that the extent of acid reflux and DGER in the anti-reflux anastomosis group was declined more intensely when compared to that in the conventional anastomosis group. Nevertheless, the varying degrees of reflux still occurred in some patients in the anti-reflux anastomosis group. It was suggested that anti-reflux anastomosis surgical procedure could be effective in controlling reflux; however, it could not prevent reflux across the anastomotic orifice. Therefore, the role of anti-reflux anastomosis in efficient control of reflux was not as optimal as the treatment for gastroesophageal reflux disease with Nissen fundoplication. Although proton pump inhibitors might decrease acid reflux in the esophagectomized patients, the use of these inhibitors could cause a 3-fold increase in the risk of esophageal adenocarcinoma. This increase, in part, might be due to an alteration in the refluxate composition created by these medications[28]. An important advantage of anti-reflux surgery is its ability to reduce all types of refluxes and this can be explained by the fact that the gastroesophageal barrier is mechanically constructed. Our previous study demonstrated that the pressure at anastomotic site measured by esophageal manometry was higher than that in the intrathoracic stomach[6]. The higher pressure at remnant esophagus (1 to 2 cm above anastomotic site) has analogous effect of the low esophageal sphincter on controlling reflux. However, the main problem associated with anti-reflux anastomosis is whether the scar in anastomotic orifice can influence the capability of controlling reflux in the future. Hence, the anti-reflux outcome of the modified fundoplication following conventional anastomosis need to be investigated for long term.

There are indications that at the molecular level, some pathways associated with inflammation and injuries are similar to those involved in carcinogenesis. Such mechanism involves the nuclear factor NF-κB pathway, in which activated COX-2 is a major downstream product. The NF-κB is a transcription factor with regulatory capacity on several genes involved in the inflammation response, and it is currently thought to play a part in gene activation involved in cancer progression[29-31]. Our study demonstrated that the COX-2 expression was present in the remnant esophagus. The up-regulation of COX-2 was similar in presence of mucosa injury in the form of reflux esophagitis or absence of any visible mucosal injury. Similar results have already been reported[8,32]. This suggested that esophageal mucosa changes did not influence the COX-2 expression in the remnant esophagus, and that the up-regulation of COX-2 might be the early episode associated with reflux since it was present in patients without endoscopic evidence of reflux-induced esophageal mucosal injury. COX-2 up-regulation was likely a more sensitive gastroesophageal reflux indicator.

Several previous studies reported that acid exposure in the distal esophagus could lead to COX-2 up-regulation in esophageal mucosa[7,33]. Other findings also demonstrated that bile acids stimulated esophageal squamous cells and Barrett's epithelial cells to trigger the expression of COX-2 and caused oxidative stress[34,35] and the ratio of hydrophobic to hydrophilic bile acids influence on COX-2 protein expression[36]. Our data also showed that higher level of COX-2 expression was detected in the postoperative patients with DGER occurred in acidic environment, especially in simultaneous acid reflux and DGER. Our findings indicated that the synergetic role in acid reflux and DGER, possibly contributed to COX-2 up-regulation. But other studies reported that the COX-2 up-regulation was induced by deoxycholic acid was partially reversed by the addition of curcumin[37]. It is a plausible idea that the additional DGER contents, including trypsin and other digestive enzymes activated by gastric acid, can induce COX-2 expression. Further studies in future may shed more light in this idea.

Earlier studies demonstrated that the increased gene expression in the distal esophagus was normalized in patients with gastroesophageal reflux disease following Nissen fundoplication[8,9]. One of the chief purposes of this study was to determine whether the modified Nissen fundoplication following esophagogastrostomy might reduce the extent of reflux and would alter the COX-2 expression level. COX-2 expression monitoring would evaluate the efficacy of anti-reflux anastomosis. Our data showed that COX-2 expression level in the anti-reflux group was significantly lower compared to that

in the conventional anastomosis group. At the time the reduced level of COX-2 expression did not return to the level detected in the normal esophageal mucosa. This result indicated that a decrease in acid reflux and DGER exposing in the remnant esophagus could down-regulate COX-2 expression even though this modified anti-reflux surgery did not completely control the reflux. A message from our study is that the monitoring of COX-2 expression might be a sensitive method to evaluate the effectiveness of anti-reflux surgery.

Conclusions

The modified Nissen fundoplication following resection AEG is effective in reducing reflux; however, it does not achieve an optimal effect on controlling the reflux. Monitoring COX-2 expression level is likely a new method to evaluate the impact of anti-reflux therapy. We reiterate that DGER occurred in acidic environment induced an increase in COX-2 expression.

Acknowledgment

The authors sincerely thank the departments of Clinical Laboratory and Pathology in our hospital making contribution to this study.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1. Liu JF, Wang QZ, Hou J. Surgical treatment for cancer of the esophagus and gastric cardia in Hebei, China. Br J Surg 2004; 91: 90-98.
2. Dresner SM, Griffin SM, Wayman J, Bennett MK, Hayes N, Raimes SA. A human model of duodenogastro-oesophageal reflux in the development of Barrett's metaplasia. Br J Surg 2003; 90: 1120-1128.
3. Wang Q, Liu J, Zhao X, Lei J, Cong Q, Li W, Li B, Wang F, Cao F, Zhang X, Zhang H, Zhang H. Can esophagogastric anastomosis prevent gastroesophageal reflux? Chin J Surg 1999; 37: 71-73.
4. da Rocha JR, Ribeiro U Jr, Sallum RA, Sallum RA, Szachnowicz S, Cecconello I. Barrett's esophagus and carcinoma in the esophageal stump (ES) after esophagectomy with gastric pull-up in achalasia patients: a study based on 10 years follow-up. Ann Surg Oncol 2008; 15: 2903-2909.
5. Aly A, Jamieson GG, Pyragius M, Devitt PG. Anti-reflux anastomosis following oesophagectomy. ANZ J Surg 2004; 74: 434-438.
6. Liu JF, Wang JD, Liu XB, Sun YH, Jiang T, Wang FS, Cao FM, Li BQ, Wang QZ. Anti-reflux anastomosis following resection of esophageal cancer. Chin J Surg 2011; 49: 61-65.
7. Hamoui N, Peters JH, Schneider S, Uchida K, Yang D, Vallböhmer D, Hagen JA, DeMeester SR, DeMeester TR, Danenberg K, Danenberg P. Increased acid exposure in patients with gastroesophageal reflux disease influences cyclooxygenase-2 gene expression in the squamous epithelium of the lower esophagus. Arch Surg 2004; 139: 712-716.
8. Oh DS, DeMeester SR, Vallbohmer D, Mori R, Kuramochi H, Hagen JA, Lipham J, Danenberg KD, Danenberg PV, Chandrasoma P, DeMeester TR. Reduction of interleukin 8 gene expression in reflux esophagitis and Barrett's esophagus with anti-reflux surgery. Arch Surg 2007; 142: 554-559.
9. Vallböhmer D, DeMeester SR, Oh DS, Banki F, Kuramochi H, Shimizu D, Hagen JA, Danenberg KD, Danenberg PV, Chandrasoma PT, Peters JH, DeMeester TR. Anti-reflux surgery normalizes cyclooxygenase-2 expression in squamous epithelium of the distal esophagus. Am J Gastroenterol 2006; 101: 1458-1466.
10. UICC, Oesophagus including oesohagogastric junction in: TNM Classification of Malignant Tumors, Wliey-Blackwell, New York, 2009; pp. 66-72.
11. Li W, Zhang ST, Yu ZL. Clinical and endoscopic features of Chinese reflux esophagitis patients. World J Gastroenterol 2008; 14: 1866-1871.
12. Johnson LF, DeMeester TR. Twenty-four-hour pH monitoring of the distal esophagus. A quantitative measure of gastroesophageal reflux. Am J Gastroenterol 1974; 62: 325-332.
13. Bechi P, Pucciani F, Baldini F, Cosi F, Falciai R, Mazzanti R, Castagnoli A, Passeri A, Boscherini S. Long-term ambulatory enterogastric reflux monitoring. Validation of a new fiberoptic technique. Dig Dis Sci 1993; 38: 1297-1306.
14. Kuramochi H, Vallböhmer D, Uchida K, Schneider S, Hamoui N, Shimizu D, Chandrasoma PT, DeMeester TR, Danenberg KD, Danenberg PV, Peters JH. Quantitative, tissue-specific analysis of cyclooxygenase gene expression in the pathogenesis of Barre's adenocarcinoma. J Gastrointest Surg 2004; 8: 1007-1016.
15. Siewert JR, Stein HJ. Classification of adenocarcinoma of the esophagogastric junction. Br J Surg 1998; 85: 1457-1459.
16. Zhang HW, Li BQ, Wang QZ. Twenty-four hours esophageal monitoring in patients with cardiac cancer postoperatively. Chin J Surg 1997; 35: 620-622.
17. Wang JD, Liu JF, Wang QZ, Tian ZQ, Zhang YF. The study of gastroesophageal reflux after esophagectomy for cancer. Chin J Thorac Cardiovasc Surg 2005; 21: 160-162.
18. Liu JF, Wang JD, Zhang SW, Tian ZQ, Wang QZ, Li BQ, Wang FS, Cao FM, Zhang YF, Li Y. Function study of the remnant esophagus and intrathoracic stomach in patients who underwent esophagectomy for cancer. Natl Med J China 2005; 85: 2678-2681.
19. Bemelman WA, Verburg J, Brummelkamp WH, Klopper PJ. A physical model of the intrathoracic stomach. Am J Physiol 1988; 254: G168-G175.

20. Procter DS. The 'ink well' anastomosis in oesophageal reconstruction. S Afr Med J 1964; 41: 187-190.
21. Demos N, Kulkarni V, Port A, Micale J. Control of post resection gastroesophageal reflux. The intercostal pedicle esophagogastropexy experience of 20 years. Am Surg 1993; 59: 137-148.
22. Yalav E, Ercan S. Reservoir and globe-type antireflux surgical techniques in intrathoracic esophagogastrostomies. Dis Esophagus 2000; 13: 282-287.
23. Qiu SX, Mao Yue, Cai HH, ZouYP, Yang DC. Study on esophageal motor function against reflux after esophagogastric anastomosis with mucosal valve. Chin J Gastrointest Surg 2005; 8: 196-198.
24. Kobayashi M, Araki K, Okamoto K, Okabayashi T, Akimori T, Sugimoto T. Anti-reflux pouch-esophagostomy after proximal gastrectomy with jejunal pouch interposition reconstruction. Hepatogastroenterology 2007; 54: 116-118.
25. Yuasa N, Sasaki E, Ikeyama T, Miyake H, Nimura A. Acid and duodenogastroesophageal reflux after esophagectomy with gastric tube reconstruction. Am J Gastroenterol 2005; 100: 1021-1027.
26. Marshall RE, Anggiansash A, Owen WA, Owen WJ. Investigation of gastro-oesophageal reflux in patients with an intact stomach: is oesophageal bilirubin monitoring a useful addition to pH monitoring? Scand J Gastrenterol 2000; 35: 904-909.
27. Vaezi MF, Richter JE. Duodenogastroesophageal reflux and methods to monitor nonacidic reflux. Am J Med 2001; 111: 160-168.
28. Lagergren J, Bergström R, Lindgren A, Nyrén O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma, N Engl J Med 1999; 340: 825-831.
29. Balkwill F, Coussens LM. Cancer: an inflammatory link. Nature 2004; 431: 405-406.
30. Barnes PJ, Karin M. Nuclear factor-kappa B: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997; 336: 1066-1071.
31. Abdel-Latif MM, O'Riordan J, Windle HJ, Carton E, Ravi N, Kelleher D, Reynolds JV. NF-kappa B activation in esophageal adenocarcinoma: relationship to Barrett's metaplasia, survival, and response to neoadjuvant chemoradiotherapy. Ann Surg 2004; 239: 491-500.
32. Wang JD, Liu JF, Wang QZ, Li BQ, Wang FS, Cao FM. The role of COX-2 and TNF-α expression in the remnant esophagus after esophagectomy. Chin Thorac Cardiovasc Surg 2012; 28: 91-93.
33. Lurje G, Vallbohmer D, Collet PH, Xi H, Baldus SE, Brabender J, Metzger R, Heitmann M, Neiss S, Drebber U, Holscher AH, Schneider PM. COX-2 mRNA expression is significantly increased in acid-exposed compared to nonexposed squamous epithelium in gastroesophageal reflux disease. J Gastrointest Surg 2007; 11: 1105-1111.
34. McQuaid KR, Laine L, Fennerty MB, Souza R, Spechler SJ. Systematic review: the role of bile acids in the pathogenesis of gastro-oesophageal reflux disease and related neoplasia. Aliment Pharmacol Ther 2011; 34: 146-165.
35. Kono K, Takahashi A, Sugai H, Souza R, Spechler SJ. Trypsin activity and bile acid concentrations in the esophagus after distal gastrectomy. Dig Dis Sci 2006; 51: 1159-1164.w
35. Takahashi Y, Amano Y, Yuki T, Mishima Y, Tamagawa Y, Uno G, Ishimura N, Sato S, Ishihara S, Kinoshita Y. Impact of the composition of gastric reflux bile acids on Barrett's oesophagus. Dig Liver Dis 2011; 43: 692-697.
36. Bower MR, Aiyer HS, Li Y, Martin RC. Chemoprotective effects of curcumin in esophageal epithelial cells exposed to bile acids. World J Gastroenterol 2010; 16: 4152-4158.