



## Cholecystectomy and Duodenogastric Reflux-: Reflux of Duodenal Content Induces Esophageal Carcinogenesis

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### Abstract

Cholecystectomy is a very common operation. Cholecystectomized patients have an increased reflux of bile and pancreatic juice from the duodenum to the stomach and a greater incidence of duodenogastric reflux (DGR). There is an increased chance for duodenal juice to enter the esophagus. Reflux of duodenal contents can induce mucosal injury and promote tumorigenesis. Therefore, we made a rat reflux model which was created a esophago-duodenostomy with total gastrectomy in order to permit chronic reflux of duodenal contents into the esophagus. Sever macroscopic, microscopic and molecular alterations were observed in nearly 100% of the cases after 40<sup>th</sup> weeks of chronic duodeno-esophageal reflux. Duodenal juice (especially, bile acid) has been found to induce the esophageal cancer in our animal model. Therefore, cholecystectomy is associated with a moderately increased risk of esophageal cancer, possibly by the toxic effect of refluxed duodenal juice (especially, bile acid) on the esophageal mucosa. We need to fully understand DGR to adequately advise patients on the effects of cholecystectomy.

**Keywords:** Cholecystectomy; Duodenogastric reflux; Esophago-duodenostomy; Esophageal cancer; Bile acids

### Introduction

Cholecystectomy is the most frequent elective abdominal surgical operation and most patients are perfectly well afterwards. Occasionally patients have symptoms of nausea and pain but these usually settle over time. In some, biliary dyskinesia is a disturbing consequence. Thankfully, for the majority with complicated biliary pathology, this is an uncommon outcome. It is particularly important to understand the physiopathology that may arise from cholecystectomy in asymptomatic patients. The aims of this work is to review if cholecystectomy further increase DGR and reflux of duodenal content induces esophageal carcinogenesis from my experimental data and literature.

### Effect of Cholecystectomy on Esophageal Microenvironment and Bile Flow

The mechanism of gallbladder function is very complex and requires many humoral and neural controls. During the interdigestive period the gallbladder is relaxed, the sphincter of Oddi is closed and the gallbladder fills with bile. Bile is stored in the gallbladder between meals and is expelled into the duodenum by contraction of gallbladder in response to meal stimulated CCK secretion from the duodenum. CCK is released in response to small peptides and fatty acids in the duodenum. It causes contraction of the gallbladder and relaxation of the sphincter of Oddi. When the gallbladder is removed, the facility for storage between meals is destroyed and bile flows continuously into the duodenum promoting retrograde reflux into the stomach leading to duodeno-gastric reflux. The loss of bile reservoir functions leads to an abnormal situation whereby bile flows into the duodenum in a continuous rather than intermittent fashion, and is related to food intake. The larger volume of bile after cholecystectomy could overload the clearing capacity of the proximal duodenum and thereby cause duodenogastric reflux through the pylorus. There is mounting evidence of the importance of duodenogastric reflux and this appears to be increased by cholecystectomy. There is evidence that implicates duodenogastric esophageal reflux in the pathogenesis of Barrett's esophagus and cancer [1,2]. Walsh, et al. [3] has previously shown that cholecystectomy is associated with an increased incidence of GERD. Freedman, et al. [4] suggested an association between cholecystectomy and an increased risk of adenocarcinoma of the esophagus, possibly due to the toxic effect of refluxed bile on esophageal mucosa. Further studies are needed regarding the link between bile reflux and esophageal carcinogenesis.

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## The Estimate of DGR

Objective physiological measurement of DGR is difficult and until recently most of the methods used for measuring DGR have required intubation, which in addition to being an invasive technique may itself promote DGR and lead to spurious results.

## Scintigraphy

Hepatobiliary scintigraphy was performed using 3-4mCi of 99m Tc-HIDA. The technique of the scintigraphic evaluation has been described in detail previously [5]. The anterior abdominal images were taken with a gamma camera, each image of 500k counts being taken at 5-min intervals for 60 min. The image interpretation and severity of DGR was assessed and graded according to the following scale; 0, no reflux; 1, reflux only into the antrum; 2, moderate reflux into the body; 3, marked reflux into the body and funds; and 4, reflux into the esophagus.

We investigated using scintigraphy in the cholecystectomy alone group. Mild DGR of grade 1 was observed in 2 (22%) of the 9 patients [6]. Scintigraphic documentation of DGR is technically easy, simple and physiologic as it is noninvasive. But the reliability and accuracy of scintigraphy has been challenged. The most common problem was the overlap of small bowel and stomach occurring in 36% of patients, which is not correctable. Other problems included overlap of the left lobe of the liver and stomach, patient movement, and the intermittent nature of bile reflux.

## Histological Determination of Bile Reflux

ERCP was performed using a TJF-240 duodenoscope (Olympus, Japan) and upper gastrointestinal endoscopy was performed with a QX10 esophagogastroduodenoscope (Olympus, Japan). During the procedure, 2 biopsies were obtained from the gastric antrum and 2 from the corpus. A pathologist (GA) who was blinded to the patient's clinical findings assessed each gastric tissue sample according to the above-mentioned Bile Reflux Index (BRI) system devised by Sobala, et al. [7] In this system, an index is derived based on the presence/severity of certain histological parameters: edema in the lamina propria, Intestinal Metaplasia (IM), Chronic Inflammation (CI), and Hp colonization in the stomach. For every specimen, the pathologist assigned a grade from 0 to 3 (representing absent, mild, moderate, or marked, respectively) for each histological parameter. An index value was then calculated using a formula derived from stepwise logistic regression analysis.  $BRI = (7 \times E) + (3 \times IM) + (4 \times CI) - (6 \times Hp)$ . According to Sobala, et al. [7], a BRI above 14 indicates DGR with 70% sensitivity and 85% specificity.

## Ambulatory Bilirubin Monitoring

In 1993 Bechi, et al. [8] validated a system for ambulatory detection of bile reflux by fiber optic spectrophotometer system (Billitec<sup>®</sup>2000, Synectics, and Stockholm, Sweden). The system consisted of a fiber optic probe of 5mm diameter with an open groove of 2 mm across which two wavelengths of light are emitted and material sampled bilirubin is detected between a mirror and the fiber optic tip at the end of the probe by light absorption at 453 nm where bilirubin has an absorption peak. Data is collected in a portable data logger and later analyzed on computer. This technique allows ambulatory detection of bile in the esophagus in much the same manner as pH detection. Nevertheless there are some limitations. Some coloured foods may interfere with the measurements and small pieces of food may get

stuck between the mirror and the tip of the fiber optic bundle. At low pH below 3.5 bilirubin forms dimmers with different optic properties causing an underestimation of bilirubin concentration of at least 30 percent [8]. The Billitec<sup>®</sup> device is the best method for monitoring bilirubin levels in the esophagus, however, interpretation of gastric bilirubin is more complex and Billitec<sup>®</sup> is not as accurate in this setting.

## Effect of DGR on Esophageal Carcinogenesis in Rat Model

Chronic Gastro Esophageal Reflux Disease (GERD) carries an increased risk for development of esophageal mucosal injury, columnar metaplasia, dysplasia and adenocarcinoma of the esophagus. These alterations are provoked by the reflux juice reaching the esophageal mucosa from the stomach which usually contains bile components. Rat experiments demonstrated that duodenal contents cause esophageal carcinoma without exposure to carcinogens, whereas gastric contents do not. To model the effect of potential carcinogenic reflux juice there are appropriate surgical methods (end-to-end esophago-duodenostomy) which provides a suitable model for reflux-induced esophageal pathologies without the need for additional carcinogen administration. The main purpose of the experimental study was to investigate the incidence of GERD induced malignant formation due to the duodeno-esophageal anastomosis.

## Animals and Design of Experimental Surgery

For my experimental GERD study 30 male wistar rats (average weight 250 g to 300 g) were used. A longitudinal midline laparotomy was performed, and an end-to-end esophago-duodenostomy with total gastrectomy (EDA) was made with 6/0 atraumatic absorbable interrupted stitches to join the duodenum to the esophagus. This procedure permits chronic reflux of duodenal contents into the esophagus through the site of the anastomosis. The rats were sacrificed 40 weeks after surgery by general anesthesia and the abdomen and thorax were opened. The esophagus and the duodenum were removed in continuity, longitudinally opened, and spread on a cork plate for macroscopic examination.

## Histopathological Changes

All animals that underwent surgery showed signs of chronic GERD, including basal cell hyperplasia, acanthosis and hyperkeratosis. The dissection of the esophageal specimen showed typical GERD features as thickened wall and marked irregular folds with a typical cobblestone appearance, extending from the mid-esophagus to the anastomosis of esophago-duodenostomy. The appearance of the larynx and duodenum was normal. In the stratified squamous epithelium of the mid-esophagus, typical reflux-associated signs were present. The lower part of the esophagus exhibited ulcerated squamous cell carcinoma with invasive foci of keratin pearls. The adjacent squamous epithelium was dysplastic, ranging from mild dysplasia to carcinoma in situ, where the basal layer was already replaced by skipping metaplasia columnar mucosa. At the squamocolumnar junction, microscopic adenocarcinoma invading the submucosal region was identified. Columnar Lined Epithelium (CLE) developed in the distal portion of the esophagus. CLE was observed in 40% at 40<sup>th</sup> week. Sever dysplasia occurred in 100%, Squamous Cell Carcinoma (SCC) was observed in 40%, and Adenocarcinoma (ADC) was observed in 30% at the 40<sup>th</sup> week. COX<sub>2</sub> immunoreactivity was mainly observed

in infiltrating cells and fibroblasts in the stroma. Esophageal cancer occurs in 2 major histopathological forms, ADC that develops from a precursor, inflammatory metaplastic lesion, the Barrett's esophagus and SCC that develops from the normal mucosa through a classical hyperplasia-dysplasia-carcinoma sequence. As compared with ADC, SCC is less frequently inflammatory, except in subjects from areas in the world where this type of cancer occurs at very high rate, such as for example Northern Iran. There were some epithelial cells of SCC and ADC which strongly expressed COX<sub>2</sub> protein [9]. Wild-type p53 protein accumulation was observed as positive nuclear staining in ADC, while it was negative in SCC. Benoit, et al. [10] proposed that chronic inflammation could represent a physio-pathological context in which the transcription factor NF-kappa B could cooperate to activate COX<sub>2</sub>. (c) The carcinogenic potential of duodenal juice reflux on the esophageal mucosa.

In humans, most of EAC arise in the Barrett's esophagus where columnar cell metaplasia replaces the native squamous cell epithelium lining the distal esophagus. It is important to note that duodenal juice, i.e. bile, induces oxidative stress in the esophageal epithelium, thereby leading to chronic inflammation and then metaplasia. In our rat model [9], total bile acid in the esophageal lumen was significantly increased in EDA model compared with the sham operated rats. Bile salts may target several potentially interconnected pathways, leading to mucosal barrier impairment, and may alter the function of cells that they are likely to contact. An understanding of the nature of regurgitation induced mucosal lesions presupposes the use of suitable experimental models. In our experiments, sever macroscopic, microscopic and molecular alterations were observed in nearly 100% of the cases after 40<sup>th</sup> weeks of chronic duodeno-esophageal reflux. We confirmed that duodenal juice without exogenous carcinogen administration can undoubtedly contribute to epithelial hyperproliferation and esophageal carcinogenesis. In a Miwa's study in rats, in whom duodeno-esophageal reflux was induced by the creation of an esophagoduodenal anastomosis was an induction of esophageal cancer that was not found if the duodenal juice was diverted further down the gut [11].

## Bile Acids

The exposure of bile acids to the epithelium of the esophagus initiates a series of reactions including increased cell permeability and dilated intercellular spaces, cell damage and repair, inflammation and activation of pro-inflammatory transcription factors and genes [12]. And all of these reactions predispose the esophagus to dysplasia and cancer [13]. Shirvani, et al. [14] suggested that bile acids stimulate COX<sub>2</sub> expression which is associated with chronic inflammation and epithelial cell growth in Barrett's esophagus and cancer. In an *in-vitro* study Jenkins found that exposure of esophageal mucosa to physiological levels of bile acids activates NF-KB, which activates COX<sub>2</sub> [15,16]. Benoit, et al. [10] proposed that chronic inflammation (refluxed duodenal contents) could represent a physio-pathological context in which the transcription factor NF-kappa B could cooperate to activate COX<sub>2</sub>.

## Conclusion

Given the increased possibility of duodenal-gastric reflux and gastro esophageal reflux after cholecystectomy, there is an increased chance for duodenal juice to enter the esophagus. Duodenal juice (especially, bile acid) has been found to induce the esophageal cancer

in our animal model. Therefore, cholecystectomy is associated with a moderately increased risk of esophageal cancer, possibly by the toxic effect of refluxed duodenal juice (especially, bile acid) on the esophageal mucosa. Therefore, we need to fully understand DGR to adequately advise patients on the effects of cholecystectomy.

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