American Medical Journal 4 (1): 127-142, 2013
ISSN 1949-0070

doi:10.3844/amjsp.2013.127.142 Published Online 4 (1) 2013 (http://www.thescipub.com/amj.toc)

Review Article; Duodeno-Gastro-Esophageal Reflux Combined and Isolated

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Received 2012-12-04, Revised 2013-01-09; Accepted 2013-05-08

ABSTRACT

Barrett's esophagus is the chief risk factor for esophageal adenocarcinoma. Reflux of gastric acid has long been related to the development of esophagitis and Barrett's esophagus, but the role of duodenal contents is controversial. We review the literature on the role of duodenal contents in the development of esophagitis, Barrett's esophagus and adenocarcinoma in addition to the role of acid suppressant therapy in the development or prevention of these changes. A computer-based search of the literature using the terms "Bilirubin, Barrett, Bile Reflux, duodeno-gastric reflux and oesophagus/esophagus" was performed. The role of bile and other constituents of duodenal refluxate were examined. Techniques for identifying non-acid reflux were also reviewed, as were the role of pH, medication and surgery in modulating disease severity. Complicated Barrett's esophagus is associated with increased exposure to gastric and duodenal refluxate. Biological effect of bile acids depends on the conjugation status, the pH of the milieu and the pKa of bile acids. While Proton Pump Inhibitors reduce the levels of DGER, they also produce changes in gastric and lower esophageal pH that activate different bile acids at different pH levels resulting in unexpected injury. Conjugated bile acids are harmful in acidic environment while unconjugated bile acids are harmful at neutral pH environment. An overlap of toxicity among conjugated and unconjugated bile acids occurs between strongly acidic and neutral pH levels. Normalisation of gastric and duodenal refluxate should ideally be the goal of treatment.

Keywords: Bile Reflux, Acid Reflux, PPI

1. INTRODUCTION

The incidence of Gastro-Esophageal Reflux Disease (GERD) increased significantly in the United States between 1970 and 1995 (Devesa *et al*., 1998). It is now the most common chronic gastro-intestinal disorder and one of the most common conditions presenting to gastroenterologists (Sonnenberg, 2004; Locke, 1997). Approximately 44% of the adult population in the United States suffer from heartburn on a monthly basis (Locke, 1997; Marshall *et al*., 1997).

Longstanding GERD is the chief risk factor for the development of intestinal metaplasia (Spechler, 1996; Cameron *et al*., 1990; Winters *et al*., 1987), which in turn was attributed to reflux of gastric acid (Balaji *et al*., 2003), but acid is not the only toxic component of the refluxate. Duodeno-Gastro-Esophageal Reflux (DGER) refers to the reflux of duodenal contents through the stomach into the esophagus. Bile acids, pancreatic enzymes and intestinal enzymes can all result in gastro-intestinal mucosal injury *in vitro* and *in vivo* (Jolly *et al*., 2004; Stein *et al*., 1999; Tibbling *et al*., 2002). There is adequate information on the toxicity of bile acids to the colonic mucosa (Owen *et al*., 1984; Turjman and Nair, 1981), hepatocytes (Scholmerich *et al*., 1984) and gastric mucosa (Gillen *et al*., 1988a; Gadacz and Zuidema, 1978), but less relating to their toxicity to esophageal mucosa (Gillen *et al*., 1988a; 1988b; Lagergren *et al*., 1999; Kauer *et al*., 1997; 1995a; Attwood *et al*., 1992). Whereas some authors consider Duodeno-Gastric Reflux (DGR) a physiological event (Schindlbeck *et al*., 1987), others believe that excessive DGR can damage the gastric

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mucosa (Stern *et al*., 1984). The evidence that DGER plays a role in the development of both Barrett's esophagus (Iftikhar *et al*., 1993; Waring *et al*., 1990) and adenocarcinoma (Miwa *et al*., 1995) is increasingly convincing. Some of the constituents of DGER can cause injury on their own while the toxicity of others is synergistic with acid at different pH values.

1.1. Duodeno-Gastro-Oesophageal Reflux

1.1.1. Pathological Reflux

As the evidence associating Esophageal Acid Exposure (EAE) to mucosal injury, Barrett's esophagus and its complications is so strong. Acid suppression has become the standard of care in the treatment of GERD. However, evidence is accumulating that Non-Acid Refluxate (NAR) plays a major contribution, whether isolated, or acting in synergy with acid reflux in causing esophageal epithelial injury.

Agents other than gastric acid must be involved in the development of esophagitis as symptoms may persist in some despite acid suppression therapy. Esophagitis occurs in patients who have undergone total gastrectomy without biliary diversion and in patients with achlorhydria (Helsingen, 1961; Orlando and Bozymski, 1973; Palmer, 2002; Yumiba *et al*., 2002; Sandvik and Halvorsen, 1988). Barrett's esophagus has been described after total gastrectomy with esophago-jejunostomy (Nishijima *et al*., 2004). This supports the view that NAR, represented by the reflux of duodenal contents alone, has a role in esophageal mucosal injury. While DGER occur in most patients following partial gastrectomy esophagitis is more prominent in patients with combined reflux (Sears *et al*., 1995).

In a Swedish study (Lagergren *et al*., 1999) of 600 patients with cancer of the esophagus or gastric cardia. Esophageal adenocarcinoma was found to be significantly associated with the severity and duration of gastro-esophageal reflux. There was an equally strong association between symptomatic reflux and the risk of adenocarcinoma in patients with Barrett's esophagus and patients without it. They also observed that patients who received medical treatment for reflux had a higher risk for esophageal adenocarcinoma than those who did not (Lagergren *et al*., 1999).

1.2. Role of pH in the Toxicity of Refluxate

Changes in the pH of the lower esophageal environment play a crucial role in altering the harmful effect of the refluxate. Kivilaakso *et al*. (1980) used three parameters to assess mucosal integrity; mucosal potential difference, tissue electrical resistance and tissue permeability to Hydrogen ions (H^+). They found that conjugated bile acids were largely responsible for mucosal injury in acidic conditions, whereas unconjugated bile acids were more critical when acid was absent. This casts helpful light on the controversy about the role of isolated and combined reflux in inducing lower esophageal injury. Stein *et al*. (1994a) evaluated 43 normal volunteers and 52 patients with GERD using ambulatory esophageal aspiration and found significantly higher concentrations of refluxed bile acids in patients with GERD ($p<0.01$). The percentage time that pH was above seven and bile acid concentration was elevated was greatest in patients with esophageal strictures and Barrett's esophagus. These findings provide strong evidence that changes in pH alter the toxicity of bile acids. Other supportive evidence comes from the development of esophagitis among ventilated patients in the intensive care unit on intravenous acid suppression therapy (ranitidine) (Wilmer *et al*., 1996). The same effect has also been reported for PPIs therapy.

1.3. Role of Duodenal Reflux in Esophagitis

The association between duodenal reflux and the severity of esophagitis was examined by Kauer *et al*. (1995b) who used a fiberoptic probe to detect bile as a marker of duodenal refluxate. They found that patients with erosive esophagitis and Barrett's metaplasia had increased exposure to duodenal juice at a normal esophageal pH. Gillen *et al*. (1988b) examined the fasting and post-prandial intra-gastric bile acids concentrations in patients with complicated and uncomplicated Barrett's esophagus, esophagitis and normal controls. They found persistently higher concentrations of post-prandial bile acids in patients with complicated Barrett's esophagus. They concluded that duodeno-gastric reflux might be implicated in the pathogenesis of complicated Barrett's esophagus. Vaezi (1995) also studied patients with and without complicated Barrett's esophagus using 24 h ambulatory pH monitoring and Bilitec© 2000 monitoring. They found the percentage time that pH was<4 and bilirubin absorbance greater than 0.14 was significantly greater in patients with complicated Barrett's esophagus. They concluded that complications of Barrett's esophagus might be related to synergism between bile and acid rather than the effect of either constituent alone. Acid and duodenal refluxate occur simultaneously in the majority of the reflux episodes and both acid and duodenal refluxate showed a graded increase across the GERD spectrum (Vaezi and Richter, 1996).

1.4. Bilirubin Exposure Time and Gradient of Injury

The observed gradient of exposure to duodenal contents in GERD appears to be independent of race. In a Japanese study the percentage time when bilirubin exposure was greater than 0.15 was significantly higher in those patients with Savary-Miller grades 3 and 4 esophagitis as compared to those with grades 1 and 2 (Osugi *et al*., 2002). It also appears that the observed relationship between bilirubin exposure and the degree of mucosal injury is independent of age. Orel and Markovic (2003) have recently demonstrated a similar pattern of exposure in paediatric reflux esophagitis. The mean esophageal acid and bilirubin exposure times showed a marked increase from normal volunteers, to GERD patients without esophagitis, to patients with Barrett's esophagus (Marshall *et al*., 2001).

Stein *et al*. (1998) studied patients from across the GERD spectrum and found that mean bile exposure time increased exponentially from patients without esophagitis, to those with erosive esophagitis to benign Barrett's metaplasia, being highest in patients with early adenocarcinoma in Barrett's esophagus. Esophageal bilirubin exposure time was highest in patients with high-grade dysplasia or early carcinoma than in Barrett's esophagus alone. Bile primarily refluxed during the post-prandial and supine periods. Pathological bilirubin exposure time (>95th percentile of normal volunteers) occurred in 11% of patients with GERD but without esophagitis, in 22% of patients with GERD and erosive esophagitis, in 55% of patients with benign Barrett's esophagus and 78.6% of patients with early adenocarcinoma or high-grade dysplasia in Barrett's esophagus. Both patients with high-grade dysplasia and Barrett's esophagus had pathological bilirubin exposure times.

Oberg *et al*. (1998) evaluated esophageal exposure to both gastric and duodenal juice in patients with both short and long segments of Barrett's esophagus. The percentage time that pH was less than 4 increased incrementally across the 4 groups from 3% in those with no mucosal injury, to 8% in patients with erosive esophagitis to 9.4% in patients with short segment Barrett's to 27% in patients with long segment Barrett's. The percentage time when bilirubin absorbance was greater than 0.2 also increased incrementally across the groups from 0.1% in those with no mucosal injury, to 4.2% in those with erosive esophagitis, to 7.9% in patients with short segment Barrett's and 15.7% in patients with long segment Barrett's esophagus.

Together these studies suggest that as acid reflux increases so does reflux of duodenal contents. The level of bile reflux closely reflects the levels of acid reflux. This relationship, combined with *in-vitro* and *in-vivo* evidence of bile acids toxicity at an acidic pH, gives a compelling suggestion that both acid and bile have a role in generating mucosal injury. The acidic environments in which bile acids are refluxed have a variable effect in accentuating their toxicity.

1.5. What we have Learned from Animal Reflux Models

Laboratory animals have been used to study the role of duodenal contents in inducing esophageal mucosal injury. Rats are used more frequently probably because they are easy to handle and faster to breed. The upper gastro-intestinal tract has been surgically altered to maximize, combine or isolate DGER to study different stimuli and effectors (Salo and Kivilaakso, 1982; 1984a; 1984b; Lillemoe *et al*., 1985).

Different laboratory animals reflux models have been used to study the role of combined and isolated duodenal reflux in lower esophageal carcinogenesis. Both pancreatic and duodenal contents can contribute to the development of adenocarcinoma in the esophagus (Pera *et al*., 1993). Induced duodeno-esophageal reflux in these models not only increased the frequency of tumour development but also affected the histology of the developed tumours, with a higher proportion of adenocarcinomas (Attwood *et al*., 1992; Nakama *et al*., 1998; Miwa *et al*., 1992a; Fujimura, 1991; Mason *et al*., 1988; Clemencon *et al*., 1984). A duodeno-esophageal anastomosis has been frequently used to study combined duodeno/Gastro-Esophageal Reflux (DER/GER), similar to but more intense than the regular DGER. Theisen *et al*. (2005) used this model to demonstrate the mutagenic effect of combined reflux through standard big blue mutagenic assay technique. They demonstrated specific mutations similar to those found in p53 mutations of human esophageal adenocarcinoma. Melo *et al*. (1999) compared DGER to isolated GOR using external carcinogen. DGER was more harmful than GOR with higher percentage of adenocarcinoma.

Nishijima *et al*. (2004) created three different models for reflux: gastrectomy plus esophago-jejunostomy, gastrectomy plus esophago-duodenostomy and gastrectomy plus Roux-en-Y anastomosis. They concluded that esophago-jejunostomy procedure does not cause regression of Barrett's esophagus but prevents the development of adenocarcinoma. These and other animal studies support the role of duodenal refluxate in

the metaplasia-dysplasia-carcinoma sequence (Gillen *et al*., 1988a; Attwood *et al*., 1992; Miwa *et al*., 1995; Nishijima *et al*., 2004; Kivilaakso *et al*., 1980; Fujimura, 1991; Clark *et al*., 1994; DeMeester *et al*., 1987; DeMeester and Ireland, 1997; Di Marco *et al*., 1990; Fein *et al*., 2000a; 2000b; Fujikawa *et al*., 1994; Gerlach *et al*., 1997; Harmon *et al*., 1978; Hofmann *et al*., 1969; Hofmann and Mysels, 1992; Hossain *et al*., 1988; Isozaki *et al*., 1995; Kauer and Stein, 2002; Kauer, 2005; Kivilaakso *et al*., 1981; Segalin *et al*., 1994; Lillemoe *et al*., 1983; 1982; Miwa *et al*., 1992b; Smallwood and Hoffman, 1976; Theisen *et al*., 2003; Vaezi *et al*., 1995; Ireland *et al*., 1996).

Manifold *et al*. (2000a) studied the role of omeprazole in gastric carcinogenesis induced by duodeno-gastric reflux. They performed a split gastro-enterostomy to induce duodeno-gastric reflux and cardiomyotomy to induce gastro-oesophageal reflux in rats. After one year 90% of rats with surgery and omeprazole developed gastric adenocarcinoma. Although none of the rats developed oesophageal cancer, oesophageal mucosal hyperplasia was more pronounced when compared to surgery alone group.

Moore *et al*. (2001) performed duodeno-esophagostomy on rats to induce duodenal reflux. They treated the study group with a daily intra-peritoneal dose of omeprazole and the control group with normal saline for 6 months. There was no difference in the number of cancers that developed in the two groups.

Using a rodent model of reflux Nasr *et al*. (2006) recently confirmed that gastric acid suppression in the presence of duodenal refluxate caused increased rates of inflammatory changes, intestinal metaplasia and molecular proliferative activity. The use of PPIs in this model suppressed acute inflammatory changes only, whereas chronic inflammatory changes persisted (Nasr *et al*., 2006).

1.6. Pathophysiology of Bile Induced Injury

The liver conjugates bile acids prior to excretion (Hofmann and Mysels, 1992; Hofmann, 1984; Hofmann and Roda, 1984; Hofmann, 1877; 1977a; 1977b). Conjugated bile acids are soluble at acidic pH. Deconjugation of these acids, however, frequently occurs in the gastrointestinal tract under the action of certain bacteria (Domellof *et al*., 1980). Upon deconjugation, these bile acids can damage the small intestinal villi (Holt, 1966) and may inhibit the transport of amino acids, sodium and glucose across the jejunal mucosa (Clark *et al*., 1969; Popesco *et al*., 1966). Cholecystectomy results in increased intestinal exposure to bile acids which increases the risk of intestinal cancer, a risk that declines down the gastro-intestinal tract with the increasing distance from the common bile duct (Freedman *et al*., 2001).

Bile acids may diffuse through intact superficial epithelial layers reaching the basal cell layer and directly induce basal cell hyperplasia (Kiroff *et al*., 1987). They may also accumulate in esophageal epithelium at concentrations up to seven times higher than the initial luminal concentration (Schweitzer *et al*., 1986). The biological effect of bile acids on esophageal epithelium depends on their conjugation status, the surrounding pH (Pera *et al*., 1993) and the pKa of the bile acids (Roda *et al*., 1995). Unconjugated bile acids promote gastric, duodenal, hepatic and colonic cancers at neutral pH (Mahmoud *et al*., 1999), where as conjugated bile acids are more harmful at acidic pH values (Katz, 2000). Before secretion into the biliary tract, the majority of bile acids are conjugated with taurine or glycine to improve their solubility. As unconjugated bile acids precipitate irreversibly in acidic environment, they cannot cross the mucosal barrier to induce damage. Conjugated bile acids, on the other hand can cross the mucosal barrier and are more toxic in an acidic environment (Richter, 2000). Barrett's esophagus patients have increased conjugated bile acids, but not unconjugated bile acids in their refluxate (Hofmann and Mysels, 1992; Gotley *et al*., 1988; Nehra *et al*., 1999).

Taurine-conjugated and glycine-conjugated acids freely soluble in water in a protonated form (Roda *et al*., 1983). This protonated form still has a lower solubility than that of its corresponding unconjugated derivative. The aqueous solubility of a number of unconjugated bile acids increases as the number of hydroxyl groups increases (Nielsen, 2005). Changes in gastric and lower oesophageal pH has a profound effect on both the concentration of bile acids and their relative toxicities, as pH has a major effect on the degree of bile acids' ionisation. The pKa value of a titratable group (bile acids) is a measure of the free energy difference between the neutral (non-ionized) and charged (ionized) state of the group (Batzri *et al*., 1991; Schweitzer and Harmon, 1986). Hence the pKa value represents capacity of ionization of a certain bile acid in a defined environment (gastric or oesophageal), which in turn reflects the amount of free H^+ ions in that environment. This is why the pKa is dependent on the pH of that defined environment. Bile acids are ionized at or below their pKa, which prevents them from crossing the mucosal barrier. When the pH value increases above the pKa value, bile acids become uncharged which allow them to freely enter the epithelial cells (Roda *et al*., 1983; Stamp, 2002; Hoffmann *et al*., 1976; Nair *et al*., 1970).

1.7. Toxicity of Bile Acids

The toxicity of bile acids has been extensively studied and shows a range of individual variation. There is considerable controversy as to which components of

the refluxate is the most damaging to the esophageal mucosa. The mechanism of mucosal damage by bile acids is poorly understood. Different hypotheses have been proposed.

Bile acids are divided into three main categories (Batzri *et al*., 1991):

- Free bile acids such as cholic acid, deoxycholic acid and chenodeoxycholic acid, have a pKa's of approximately 7
- Glycine conjugated bile acids such as Glycholic acid, glydexoycholic acid and glychenodexycholic acid have a pKa's of 4.3-5.0
- Taurine conjugated bile acids such as taurichollic acid, taurideoxycholic acid and taurichenodeoxycholic acid have a pKa's of <2

Batzri *et al*. (1991) described bile acids as strong detergents, capable of disrupting lipid bi-layers of the GI tract epithelium and altering its permeability. They can then enter the epithelial cells and become partitioned between the cytoplasm and the lipid membrane compartments, causing disruption of cellular function. Schweitzer *et al*. (1984) reported a positive correlation between accumulation of bile acids within the mucosa and the degree of mucosal injury. They also found significant disruption of rabbit esophageal mucosa at bile acids concentration well below those required to solubilise phospholipids. This evidence opposes Batzri's concept of bile salt damage being mediated by their detergent properties.

Using net flux of hydrogen ion permeability as a measure of disruption, taurocholate was shown to produce disruption in both extent of mucosal barrier disruption and bile salt absorption in relation to changes in bile salt concentration and in pH of the rabbit esophageal epithelium (Salo and Kivilaakso, 1984a; 1984b). Demonstrated that both taurocholate and lysolecithin disrupt the trans-mucosal potential difference of rabbit esophageal mucosa in an acidic environment.

1.8. Toxicity of Pancreatic Secretion

Pancreatic secretions are also harmful. Pancreatic amylase which, loses its activity at pH of ≤2.0 in the stomach, normally gains activity again at a pH of 7.0 in the esophagus (Evander *et al*., 1987). Trypsin, a pancreatic enzyme similar in action to pepsin, causes damage to the esophageal mucosa by its proteolytic ability. It is most active at a slightly alkaline pH (7.6-8.0) and loses more than 50% of its activity in pH less than 5.0 (Mud *et al*., 1982). In surgically induced animal models, Trypsin in the oesophageal refluxate may be as high as 12U/mL (Imada *et al*., 1999). The level of Trypsin is low in human gastro-esophageal refluxate, sometimes even undetectable. While it is barely harmful in acidic environment, it is more harmful in acid suppressed situations as post-gastrectomy or during acid blocking therapy (Imada *et al*., 1999). Its inhibition in post-gastrectomy model resulted in both effective prevention and treatment of esophagitis (Imada *et al*., 1999).

1.9. Measurement of Reflux

1.9.1. Is Alkaline Reflux Indicative of DGER?

The study of DGER had been hindered by inadequate monitoring systems. The measurement of esophageal pH as an indication of DGER has a limited role. Pellegrini *et al*. (1978) using 24 h pH monitoring demonstrated the importance of alkaline reflux (pH ≥7) as being a marker for DGER. However studies combining bilirubin absorbance and pH monitoring have shown that the term 'alkaline reflux', as being indicative of duodenal reflux, is a misnomer. Factors such as diet, periodontal disease, the pooling of saliva by strictures and the increased secretion of saliva all contribute to technical inaccuracy of isolated pH monitoring. Other studies (Mattioli *et al*., 1990) have suggested that the most common reason for the esophageal pH to increase to a level higher than 7 is the secretion of bicarbonate by the sub-mucosal glands of the esophagus.

Mattioli *et al*. (1990) confirmed that 24-h ambulatory esophago-gastric pH monitoring using a triple pH probe (placed at the distal esophagus, the fundus and the antrum of the stomach) is a reliable and well-tolerated technique for detecting duodenal reflux. Just *et al*. (1996) studied the pH changes associated with DGR. They found that rises in intra-gastric pH do not predict the presence of bile in normal subjects as DGR does not cause major alkaline shifts of intra gastric pH. They concluded that measuring "alkaline reflux" with ambulatory intra-gastric pH monitoring alone is an outdated technique. They recommended that Bilitec$^{@}$ 2000 should become the standard technique for the detection of intra-luminal bile.

1.10. Esophageal Aspiration Studies

Esophageal aspiration studies have shown that bile acids are found in aspirates of normal volunteers indicating that bile may be present in the stomach and esophagus of normal people without any apparent symptoms aspirates (Kauer *et al*., 1997). Nevertheless, isolated bile or acid reflux into the esophagus of patients with intact stomach is uncommon (Gotley *et al*., 1988).

Nearly half of all patients with reflux symptoms have combined acid and bile reflux (Iftikhar *et al*., 1993). The combined effect of both gastric and duodenal juices causes severe esophageal mucosal damage in patients with GERD. The vast majority of duodenal reflux injury occurs at a pH range of 4 to 7 (Stein *et al*., 1994b), at which an overlap in the activity of bile acids, the major components of duodenal juice, are capable of damaging the esophageal mucosa (Bechi *et al*., 1993).

1.11. Measurement of Bile Reflux

Esophageal aspiration studies utilizing High Performance Liquid Chromatography (HPLC) have been used to identify the most common bile acids in the esophagus. Kauer *et al*. (1997) detected bile acids in the aspirates of 58% of normal subjects and 86% of patients with reflux disease ($p<0.003$). They also demonstrated a higher bile acid reflux rate in GERD patients and confirmed that the predominant bile acids refluxed are glycine conjugates. Gotley *et al*. (1990) using aspiration studies and HPLC identified predominantly conjugated bile acids in 87% of patients with reflux. The highest levels of bile acids were found in the supine periods and in eleven of 45 patients, levels exceeded 200 μ moL^{-1}. The median conjugated bile acid concentration during the daytime period was significantly lower than that seen at night time and was less than 20 μ moL^{-1}.

Iftikhar *et al*. (1993) using 18-hour stationary aspiration and HPLC showed that the concentration of bile acids in patients with Barrett's metaplasia was significantly higher than in normal subjects. Taking the 95th percentile of controls as the upper limit of normal, 20% of patients with esophagitis and 50% of patients with intestinal metaplasia had an increased exposure to bile acids. The median concentration of total bile acids aspirated in patients with Barrett's esophagus was 1351 μ moL^{-1} compared to 465 μ moL^{-1} in patients with uncomplicated GERD. They concluded that bile acid reflux was implicated in the progression of disease severity. Stein *et al*. (1995) evaluated 43 normal volunteers and 52 patients with GERD using ambulatory esophageal aspiration and found significantly higher concentrations of refluxed bile acids in patients with GERD ($p<0.01$). The percentage time that pH was ≥ 3 and bile acid concentration was elevated was greatest in patients with esophageal strictures and Barrett's esophagus.

1.12. Spectro-photo-metric Bilitec Studies

Stationary aspiration studies pointed to a graduated level of injury with progressive exposure to bile acids. This has been confirmed in subsequent ambulatory spectro-photo-metric Bilitec studies. The introduction of the Bilitec Probe ^{TM} that detects the presence of bilirubin, the most common bile pigment, in the esophagus as a surrogate marker for duodenal reflux has been a major advance (Shay *et al*., 2004). It relies on the recognition that increased absorption of light at bilirubin characteristic wavelength (450 nm) correlates well with the presence of bilirubin and hence bile in the esophagus. It is used as a 24-h ambulatory device, is relatively non-invasive and is independent of the problems associated with pH monitoring.

Introduction of the Bilitec probe has greatly facilitated investigating the role of DGER in the spectrum of injury associating reflux disease, Barrett's esophagus and their complications. The Bilitec spectro-photo-metric technique was initially validated by *in-vitro* studies (Marshall *et al*., 1997). Recent *in-vivo* study of the sensitivity of the Bilitec probe has been proven to be more reliable with small number of false positive (Kauer *et al*., 1995a). Introduction of gastric aspirate into the esophageal lumen after *in-vitro* validation studies of the aspirates has shown that the Bilitec probe may be less sensitive *in-vivo* in detecting significant reflux episodes (Stein *et al*., 1995). It appears to underestimate the presence of duodenal juice at acidic pH, (by at least 30% in acidic medium $pH<3.5$) and requires a modified diet to avoid interference with readings and subsequent false positives (Fein *et al*., 1996). Despite the variance in validation study findings, the probe is accepted as being sufficiently accurate for ongoing use in clinical studies.

The currently accepted absorbance threshold for esophageal bile reflux is greater than 0.14 (Stipa *et al*., 1997; Barrett *et al*., 2000). However there has been criticism of the use of the 0.14 level of absorbance, as the original validation studies continued to show variance and a non-linear progression at an absorbance level of between 0.14 and 0.20 (Sifrim *et al*., 2004). Kauer *et al*. (1995a) in their study preferred the higher value of 0.20 on the basis that below this value absorbance was not necessarily due to bilirubin. Other studies have utilized different absorbance thresholds based on their own validation standards. Stein *et al*. (1998), consequent to their own validation studies, used an absorbance threshold of 0.25. A similar level was also employed by Okholm *et al*. (1999) to take account of possible interference in absorbance by dietary intake. The 0.14 level of absorbance is the most widely used in studies to date. Establishing a universally accepted standard level of absorbance is important to avoid generating a large volume of essentially incomparable data.

1.13. Recent Advances in Measurement

A newly introduced technology for detecting the type of refluxate in the lower esophagus is the intra-luminal impedance monitoring technique that detects the distribution, composition and clearing of both acid and non-acid esophageal reflux. Using electrodes mounted

on a standard esophageal pH-monitoring catheter, it allows differentiating between liquid, gas and combined liquid and gas. It detects reflux events regardless of their pH, measuring the amount of time refluxed, material remains in contact with the esophageal, mucosa and the distance above the LES to which the refluxate enters the esophagus (Cabrol *et al*., 1990; Lujan-Mompean *et al*., 1993; Lorusso *et al*., 1990).

Intra-luminal impedance monitoring is considered the only recording method that can achieve high sensitivity for detection of all types of reflux episodes. Its use in combination with pHmetry improves the capacity to detect all reflux events providing the best possible evaluation of the function of the anti-reflux barriers (Jazrawi *et al*., 1993).

The diagnostic yield of combined pH-impedance monitoring was used to compare the role of non-acid reflux in the pathogenesis of reflux-related symptoms between individuals on or off PPI therapy (Zerbib *et al*., 2006). More than half (55%) the individuals off PPI therapy had positive symptoms association probability of which 31.1 4.1 and 20.3% has occurred during acid reflux, non-acid reflux and mixed reflux (acid and non-acid) respectively, illustrating that their symptoms were mostly relevant to acid and mixed reflux than non-acid reflux. For individuals on PPI therapy, 36.7% had positive symptoms association probability of which 5, 16.7 and 15% has occurred during acid reflux, non-acid reflux and mixed reflux respectively, illustrating that their symptoms were mostly relevant to non-acid and mixed reflux.

Combined multi-channel intra-luminal impedance and pH monitoring (MII-pH) currently represents a change in the reflux testing paradigm with its capacity to detect both liquid and gas reflux and to differentiate between, acid, non-acid and mixed reflux (Tutuian and Castell, 2006). It is becoming the new gold standard method of testing patients with persistent symptoms on acid suppression therapy (Castell and Tutuian, 2007; Bredenoord *et al*., 2007).

The only downside of MII-pH monitoring is that it cannot confirm or exclude the presence of bilirubin in non-acid and mixed reflux. A down side that renders Spectro-photo-metric Bilitec Study more superior than MII-pH in detecting bile reflux but less useful in detecting gas reflux.

1.14. Control of Reflux

1.14.1. Role of Surgery in the Control of DGER

During the 1930 sec hiatus hernia was recognised by the medical community as a significant problem. In the 1940 sec it was found to be associated with esophagitis. It was Philip Allison who was first to associate the symptoms of hiatus hernia to the occurrence of GER. He initiated the modern era of anti-reflux surgery by introducing the Allison repair operation. Ronald Belsey then introduced the partial fundoplication (Belsey repair). Rudolph Nissan performed his fundoplication first in 1937 for a bleeding chronic ulcer of the distal esophagus and again in 1946 for an intra-thoracic stomach. It was until 1954 when he adjusted this operation (fundoplication without resection of the cardia) as a treatment for GERD (Jazrawi *et al*., 1993; Rothwell *et al*., 1997).

Stein *et al*. (1998) compared the role of surgery with medical treatment in controlling esophageal bile reflux. They showed that esophageal bile exposure was reduced by medical treatment (20 mg of omeprazole twice daily) from 16.2% of total monitoring time without acid suppression to 8.9% with acid suppression. Laparoscopic Nissen's fundoplication however normalized esophageal acid and bile exposure in all but 1 of 16 patients who volunteered for follow up reducing esophageal bile exposure from 16.4% pre-operatively to 2.4% bilirubin exposure time post operatively. They concluded that Nissen's fundoplication prevents bile reflux into the esophagus which medical acid suppression alone cannot achieve.

Mainie *et al*. (2006) studied the efficiency of Nissen's fundoplication in the treatment of patients with persistent reflux symptoms documented by (MII-pH) monitoring despite acid suppression therapy. After a mean follow-up of 14 (7-25) months post laparoscopic Nissen's fundoplication, they concluded that patients with positive symptom index resistant to PPIs with documented acid or non-acid reflux by MII-pH monitoring can be treated successfully by laparoscopic Nissen's fundoplication.

Several studies have shown that DGR as well as GER (Freedman *et al*., 2001) increases after cholecystectomy (Manifold *et al*., 2000a). It has been suggested that the effect of cholecystectomy is mediated by compromising the LES function (Vela *et al*., 2001). McDonnell *et al*. (2002) suggested that the compromise in LES function may be related to elevated levels of cholecystokinin released in patients post cholecystectomy in response to a meal stimulus. The increase of DGR following cholecystectomy may be greater in patients who are symptomatic (Wurm and Caestecker, 2003). In a population-based cohort study of cholecystectomized patients in Sweden between 1965 and 1997 cross-linked with the Swedish Cancer Register, Freedman *et al*. (2001) found that cholecystectomy was associated with a moderately increased risk of adenocarcinoma of the

esophagus. They also found that increased intestinal exposure to bile acids after cholecystectomy increases the risk of intestinal cancer, a risk that declines with the increasing distance from the common bile duct. In contrary Manifold *et al*. (2000b) concluded after performing bilirubin and pH monitoring on 17 patients with gallstones pre and post cholecystectomy that cholecystectomy does not result in increased DGR or GER.

A malfunctioning gallbladder could behave as an absent gallbladder as in cholecystectomy. Nasr *et al*. (2006) studied gallbladder function in patients with uncomplicated Barrett's esophagus and esophageal adenocarcinoma. The mean gallbladder ejection fraction decreased progressively from controls to Barrett's to adenocarcinoma and was significantly lower in Barrett's group (60.9%; $p = 0.019$) and adenocarcinoma group (47.9%; $p<0.001$) compared with normal controls (70.9%). They concluded that gallbladder malfunction increases DGER, exposing the lower esophagus to an altered chemical milieu which, in turn, may have a role in promoting metaplasia-dysplasia-neoplasia sequence in the lower esophageal mucosa.

1.15. Role of Medication in the Control of DGER

Burget *et al*. (1990); Howden and Hunt (1990) and Armstrong *et al*. (1988) investigated the association between symptoms and acid or Non-Acid Reflux (NAR) in a small subset (n = 5) of patients with heartburn and found that heartburn associating NAR decreased dramatically with omeprazole treatment (71 Vs 10%). This decrease in heartburn was offset by an increase in regurgitation symptoms during acid reflux (20 Vs 67%) during therapy and non-acid reflux (26 Vs 88%) during therapy. They concluded that although GERD symptoms are more common with acid reflux, they do occur with NAR.

The last twenty years have seen the evolution of many improved strategies in the medical treatment of GERD and Barrett's esophagus (Chiba, 1997; Stevens *et al*., 2001). Current medical treatment almost universally involves aggressive acid suppression in order to reduce mucosal injury. They afford resolution of symptoms using lifelong PPIs. Antacids and H_2 receptors antagonists no longer have a major role in the treatment of Barrett's oesophagus (Ireland *et al*., 1996).

Acid suppression has complex effects on the intra-gastric milieu. Patients on long-term omeprazole have decreased acid secretion and an elevated gastric pH. The resultant overgrowth of the duodenal and gastric microflora (Moore *et al*., 2001; Wetscher *et al*., 1999a; 1999b) may facilitate the deconjugation of bile acids in the stomach. This in turn may increase the concentration of dehydroxylated and more toxic unconjugated bile acids (Menges *et al*., 2001).

Acid suppression therapy can increase gastric pH from 2 to approximately 6.5. A $pH\leq4.0$ is considered to be the threshold for acidic reflux (Menges *et al*., 2001). The increase in pH potentially causes deconjugation and release of the more noxious free bile acids. While numerous studies and meta-analyses have confirmed the superiority of PPIs over H_2RAs for the healing of erosive esophagitis, few studies have examined the role of acid suppression by Proton Pump Inhibitors (PPIs) or H_2 Receptors Antagonists (H_2RAs) in duodenal reflux-induced esophageal mucosal injury (Champion *et al*., 1994).

It has been suggested that the introduction of acid suppression therapy correlates with the rapid increase in incidence of esophageal adenocarcinoma in many Western countries. Marshall *et al*. (1998) reported a progressive increase in the prevalence of esophageal adenocarcinoma in a duodenal reflux animal model as the level of gastric juice in the refluxate was reduced. They concluded that the presence of gastric juice in refluxed duodenal juice protects against the development of esophageal adenocarcinoma. They hypothesised that continuous profound acid suppression therapy may encourage esophageal metaplasia and tumorigenesis in patients with DGER. Several animal models examined the effect of PPI therapy on DGER. Moore *et al*. (2001) found that the addition of omeprazole did not increase the number of esophageal adenocarcinomas in animals that underwent different forms of surgical reflux to maximize DGER (Netzer *et al*., 2001). In contrary Nasr *et al*., 2006) demonstrated that the use of acid suppression therapy in the presence of duodenal refluxate caused increased rates of inflammatory changes, intestinal metaplasia and molecular proliferative activity. They also found that PPIs suppressed acute inflammatory changes only, whereas chronic inflammatory changes persisted which progressed to Barrett's esophagus (Nasr *et al*., 2006).

Manifold *et al*. (2000a) analysed the correlation between acid and biliary reflux in patients with esophagitis and patients with Barrett's esophagus by performing 24-h pH and bile reflux testing utilizing a Bilitec probe. They also examined the effects of PPIs in 20 patients with esophagitis and 23 patients with Barrett's esophagus. Patients off medication that could affect acid secretion or GI motility were studied and compared to patients on PPI therapy. They found that Barrett's patients have increased acid and bile exposure. The median time that bilirubin absorbance was >0.2 was 12.8% in patients with esophagitis and 34.7% in patients

with Barrett's esophagus. Within the esophagitis group the median percentage time that Bilirubin absorbance was > 0.2 was 6.9% in grades I and II and 18.0% in grades III and IV. They noticed that the gradient of damage seemed to correlate with the degree of bilirubin exposure. On treatment with a proton pump inhibitor, esophageal bilirubin absorbance decreased from 29.8 to 0.7% in the Barrett's group and from 21.5 to 0.9 % in patients with esophagitis. They concluded that there was a good correlation between the duration of the esophageal acid/bile exposure and the severity of the pathological change in the esophagus. This study has the advantage of using bile absorbance as indicative of duodenal reflux which is more specific than using only suggestive measures as pH or MII-pH monitoring which only use non-acid reflux as indicator of duodenal reflux (Wetscher *et al*., 1999a).

Champion *et al*. (1994) treated 9 patients (3 with GERD and 6 with Barrett's esophagus) with 40 mg of omeprazole daily and reported a reduction in the percentage time bilirubin absorbance >0.14 from 32.8 to 4.7%. These results showed that omeprazole reduced esophageal bile exposure but not to its normal range. Marshall *et al*. (2001) examined the effect of omeprazole 20 mg twice daily on duodeno-gastric and gastro-esophageal bile reflux in Barrett's esophagus and found that esophageal bilirubin exposure was reduced from a median of 28.9 to 2.4%.

Triadafilopoulos (2006) reported a significant reduction in bile reflux after 28 days of treatment with 40mg/day of pantoprazole. They also noted that patients who were Helicobacter pylori positive had significantly higher bile reflux times than patients who are H. pylori negative. In contrast, Scarpignato *et al*. (2006) reported that acid suppression therapy with omeprazole 20 mg twice daily had little effect on the levels of DGR in either patients with Barrett's esophagus, or in healthy controls.

Acid suppression leads to bacterial overgrowth (Ouatu-Lascar and Triadafilopoulos, 1998; Katzka and Castell, 1994; Basu *et al*., 2002), increase in gastric pH and reflux of toxic unconjugated bile acids known to cause lower oesophageal injury. Bile reflux is more sever in patients with long segment Barrett's oesophagus and aggressive acid suppression therapy reduces both acid and bile reflux. It is unclear why bile reflux should reduce in response to PPIs but Richter *et al*. (2000) suggest that this is related to the reduced volume of acid in the stomach and so reducing the volume available for reflux into the esophagus. Incomplete acid suppression allows oesophageal exposure to bile acids to continue and may potentiate the ability of bile acids to cause damage, this may be a risk factor for adenocarcinoma. The demonstration that DGER is not affected by omeprazole therapy by some research groups does support the trend of monitoring patients on long term PPIs for signs of oesophageal neoplasia. Different studies showed that symptoms resolution in Barrett's patients on acid suppression does not guarantee acid reflux control.

2. CONCLUSION

Bilirubin exposure is increased in a stepwise manner across the spectrum of GERD. Complicated Barrett's esophagus is associated with the highest duodenal refluxate exposure. Bilirubin exposure is merely a surrogate marker for the complex mixture of bile acids and enzymes found in the duodenal refluxate, some of which are more toxic in the presence of acid than alone. The toxicity of specific bile acids and enzymes varies with the pH of the refluxate. Medical therapy with PPIs appears to significantly reduce the levels of DGER but fail to stop oesophageal mucosal injury. Concern exists that the changes in gastric and lower esophageal pH created by the use of PPIs medications may activate different bile acids at different pH levels and result in unexpected injury. It is wise enough to rule out associated bile reflux using a reliable technique such as Bilitec$^{@}$ 2000 or MII-pH monitoring in patients with GERD before commencing them on long-term acid suppression therapy. Surgical treatment by Nissen's fundoplication is proven to be an effective treatment in reducing DGER to normal levels. The normalisation of both the reflux of acid and duodenal contents should be the goal of treatment.

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