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Original Article

The Columnar-lined Mucosa at the Gastroesophageal Junction in Non-Human Primates

Carlos A. Rubio[1*], Edward J. Dick Jr[1], Natalia E. Schlabritz-Loutsevitch[2], Abiel Orrego[1*] and Gene B. Hubbard[1]

[1]Southwest National Primate Research Center at the Southwest Foundation for Biomedical Research, San Antonio, TX, and [2]Department of Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, TX 78245-0549, USA

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Abstract: Despite that anatomists consider the *cardia* as a portion of the stomach, there is disagreement in the literature over whether the *cardia* mucosa, described as columnar-lined with mucus-producing glands (CLMMG) with or without occasional interspersed oxyntic cells, is part of the stomach, part of the esophagus or a distinct entity. For some authors this mucosa phenotype is a metaplastic glandular change of the distal esophagus caused by protracted gastro-esophageal reflux (GER). In this survey, the presence of CLMMG mucosa was searched for at the esophagus-gastric junction in 50 non-human primates (NHP). The length of the CLMMG (between the squamous epithelium of the esophagus and the first oxyntic fundic gastric gland) was assessed by the aid of an ocular microscale. In all three foetuses, all four stillborn baboons and one 4 day old baboon, the columnar-lined mucosa showed depressions that corresponded to early epithelial pits without glands. In the remaining 45 post-natal NHP, the length of the CLMMG mucosa varied from 0.8 mm to 25.2 mm, and the CLMMG mucosa had replaced the distal esophageal squamous epithelium. The size was neither influenced by the post-natal age nor by the gender of the animals. In NHP, regurgitation with rumination is a natural physiological process leading to GER. The present investigation substantiates the notion that the columnar-lined mucosa with mucus-producing glands is a post-natal developmental process in NHP. These animals seem to offer an excellent spontaneous model to study the series of histological events that take place in the distal esophagus of NHP, most likely under the influence of protracted GER.
Key Words: Metaplasia, esophagus, gastroesophageal junction, reflux, non-human primates

Introduction

Anatomically, the esophagus begins in the neck at the cricoid cartilage, passes through the thorax within the posterior mediastinum and extends past the diaphragm to its junction with the stomach [1]. The stomach is an intra-abdominal organ usually divided into four regions: the cardia (*antrum cardiacum,* from the Greek *kardia*: heart), the fundus, the body and the antrum. The cardia is provided with a sphincter called the cardiac sphincter, or the gastroesophageal sphincter or most commonly, the lower esophageal sphincter (LES) [1, 2]. At the histological level, the esophagus is covered by nonkeratinizing stratified squamous epithelium, the *cardia* by columnar lined mucosa having mucous secreting glands with or without occasional oxyntic cells and the gastric mucosa by fundic oxyntic glands [1-3].

The presence of the *cardia*c mucosa (CM) at birth [1, 4] is still much debated in the literature [5-12]. Some authors claim that the CM is a normal histological component in newborns [3-5]. Others maintain that at birth, the squamous epithelium of the esophagus and the oxyntic mucosa of the proximal stomach meet at the gastroesophageal-junction region [4,13-16] and that CM evolves following the damage caused by protracted regurgitation (reflux) of gastric juices of low pH into the lower end of the esophagus [17].

*Present address: Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden.

In a recent critical review of the literature, CM was found in about 50% of the general population [18]. Chandrasoma *et al* [18] recorded CM in 26% (n=19) of 72 retrospective autopsies. The same authors analyzed the entire circumference of the distal esophagus in 18 prospective autopsies. Partial CM was found in 44% (8/18) of the cases. CM has been found in 35-81% of the patients studied [19-22].

The majority of the individuals in a normal population occasionally complain of heartburn, a symptom also known as cardialgia or pyrosis. The prevalence of heartburn is high in the West (40% among Swedes [23], 34% among Germans [24] and in the US 36% individuals complaining of heartburn at least one a month [25]). In a study of 597 Caucasian British patients with dyspepsia, 72% had heartburn symptoms [26].

For obvious reasons, heartburn is not a symptom that can be easily identified in non-human primates (NHP). These animals, however, regurgitate partially digested food and chew it again through rumination [27]. Thus, regurgitation with rumination is a natural physiological process in NHP [28], most likely leading to gastro-esophageal reflux (GER).

In previous work we studied the histology of the squamous epithelium of the distal esophagus in NHP [29]. It was found that the squamous epithelium was normal in some animals while others showed inflammatory changes such as reflux esophagitis or lymphocytic esophagitis, the latter a non-GER condition characterized by lymphocytic infiltration of the squamous epithelium, recently described in humans [30].

More recently, while reviewing histological sections from the entire esophageal-gastric region in NHP, it became apparent that the distal esophagus in some animals was lined by columnar epithelium having mucus-secreting glands, thus resembling CM in humans. The purpose of this work was to review a cohort of NHP having histological sections of the entire esophageal-gastric region. To by-pass the confusing terminology in the human literature regarding the *cardiac* mucosa, that mucosa in NHP will be referred to as columnar-lined mucosa with mucous glands (CLMMG).

Material and Methods

Animals

The distal esophagus together with the proximal stomach was removed *en block* at autopsy in 50 NHP dying from natural causes: 47 baboons (*Papio* spp.), one marmoset (*genus Callithrix*), one Cynomolgus macaque (*Macaca fasciculate*) and one spider monkey (*Ateles* spp.). The NHPs were members of colonies at the Southwest National Primate Research Center, Southwest Foundation for Biomedical Research. The conditions of animal housing have been reported elsewhere [31]. Briefly, the NHP were housed in metal and concrete indoor-outdoor cages and were fed commercial monkey diets, occasionally supplemented with a variety of fruit and vegetables. Water was available *ad libitum.*

Esophago-gastric longitudinal tissue samples were fixed in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 µm, stained with hematoxylin and eosin (H&E) and evaluated by light microscopy. All procedures were carried out in accordance with the Institutional Animal Care and Use Committee guidelines.

Histological Definitions and Examination

Primordial Mucosa: According to some authors [5–6] two columnar epithelial types can be discerned between the squamous epithelium of the esophagus and the parietal cells in the gastric mucosa of embryos and foetuses: "primitive oesophageal mucosa" and "primitive stomach mucosa". The "primitive oesophageal mucosa" is characterized by ciliated epithelium that disappears at 24 weeks [32]. The "primitive stomach mucosa" is composed of a layer of flat columnar cells containing depressions that correspond to early gland pits distally [5–6, 32].

Mature Mucosa: i) Stratified squamous-cell mucosa. The distal esophagus is covered by stratified squamous epithelium showing discrete papillae with one layer of basal cells and none to occasional intraepithelial lymphocytes [33]. *ii) Collumnar-lined metaplasia with mucous glands* (CLMMG). The distal esophagus is covered by metaplastic columnar epithelium either with mucous-producing glands, or mucous-producing glands admixed with occasional oxyntic cells. *iii) Fundic gland mucosa.* The pits of the fundic mucosa of the stomach, lined with columnar

epithelium, occupy less than one quarter of the mucosal thickness. The glands are tightly packed and straight rather than coiled as in the CLMMG. They have 3 portions [1]: the basal portion where chief cells predominate, the isthmus where parietal (oxyntic) cells predominate, and the neck region where mucin-producing neck cells may be found admixed with parietal and chief cells. It should be pointed out that the fundic mucosa does not include glands or group of glands with mucous-producing cells, with or without occasional oxyntic cells.

Assessment of the Length of CLMMG

The distance between the distal end of the squamous epithelium of the esophagus and the first fundic gastric gland found, was recorded in mm by the aid of an ocular microscale in all animals. The size of the *CLMMG* was noted as 0 when the following mucosal settings were present: *i)* The squamous epithelium of the esophagus met the first fundic gland, *ii)* The squamous epithelium of the esophagus met the columnar mucosa showing depressions corresponding to gastric pits without glands, and *iii)* The squamous epithelium of the esophagus met columnar cells showing depressions corresponding to gastric pits with neck cells without mucous-producing glands (with or without occasional oxyntic cells).

Statistical Analysis

The size of GLMMG in mm and the age of the animals were tested using a nonparametric correlation coefficient test (Spearman R). The nonparametric test of Wilcoxon was used to compare the size of the GLMMG and the gender of the animals. Statistical significance was defined as $p<0.05$.

Results

Age and Length of the CLMMG

The mean age in 46 NHP was 11.7 years (range 0-27 years). The age in the remaining four NHP was unknown.

In all three foetuses, all four stillborn baboons and one 4 day old baboon, no CLMMG could be detected and the CLMMG was recorded as 0 mm (see **Materials and Methods**). The squamous epithelium met directly the fundic mucosa in two of the four stillborn baboons and in the baboon 4 days of age. In the remaining 38 NHP ages 20 days or older, the mean size of the CLMMG was 8.9 mm (range 0.8 - 25.2 mm) (**Figure 1**).

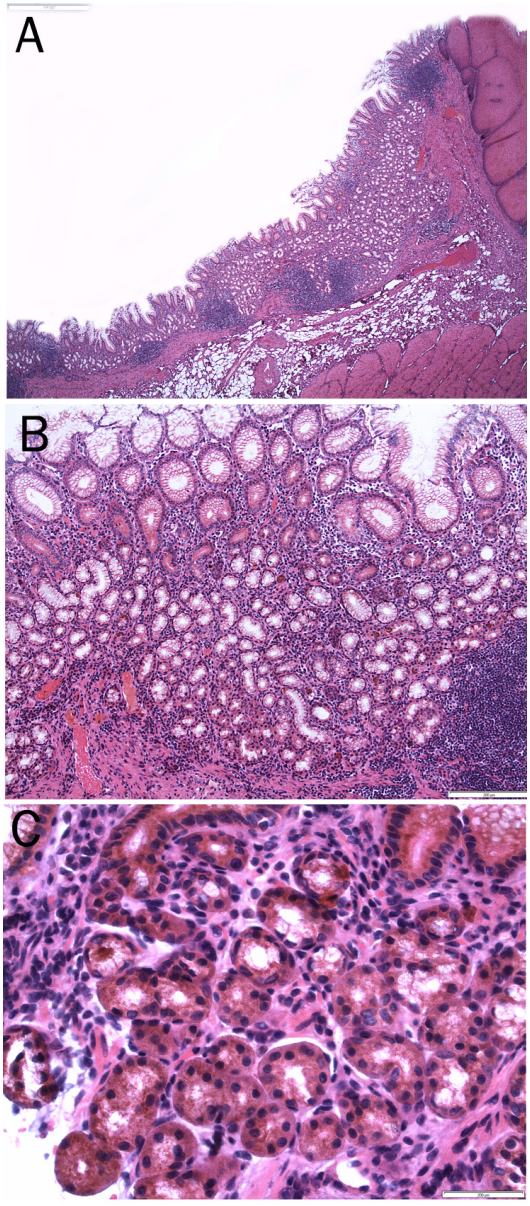

Figure 1 **A.** Extended columnar-lined mucosa with mucous glands (CLMMG) in a 12 years old baboon. Note the squamous epithelium on the right (H&E, 4x). **B.** Higher power view demonstrating the columnar-lined mucosa with mucous glands (H&E, 10x). **C.** Oxyntic fundic mucosa found at the left end of panel A (H&E 25x).

As shown in **Figure 2,** there was no correlation between the age in the 38 NHP and the length of the CLMMG (correlation coefficient: 0.27).

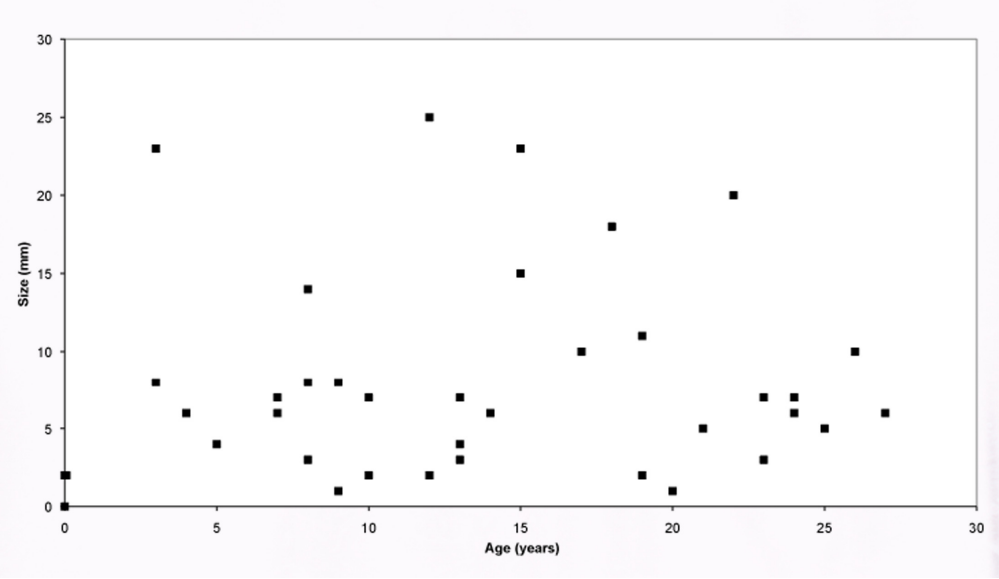

Figure 2 Correlation between age of the NHPs and the size of CLMMG mucosa.

Gender and Length of the CLMMG

The gender was known in 46 of 50 NHPs with 24 males and 22 females. The mean age in males was 11.0 years (range 0-25 years) and in females 12.9 years (range 0-27 years). The difference in age between males and females was non-significant ($p<0.6$).

In males the mean CLMMG size was 6.3 mm (range 0 - 19.5 mm) and in females 8.5 mm (range 0 - 22.7 mm). Although a trend of a longer CLMMG was found among female NHP, the difference was non-significant ($p<06$).

Discussion

Many authors claim that the *cardia* mucosa is present at birth [5, 6, 8, 10, 12, 34, 35]. Other authors have challenged this notion [14-18, 35-42]. The controversy in recognizing the CM in humans as a separate histological compartment seems to have originated from the fact that the *cardia*, a term defining an anatomical part of the stomach [1] has been extrapolated to label a mucosa phenotype that according to many authors does not exists at birth [35, 41]. Despite disagreement in the literature over whether the *cardia* is part of the stomach, part of the esophagus or a distinct entity, the majority of the anatomists maintain that the *cardia* is a portion of the stomach surrounding the cardio-esophageal junction [1, 2]. Consequently, tumors of the *cardia* region are today usually coded with those in the stomach. On the other hand, some pathologists claim that many carcinomas, initially classified as cancers of the gastric *cardia*, are in reality esophageal cancers [13, 37, 40]. The ongoing discussion regarding the identity of the CM in humans, namely whether this mucosa is a normal histological component of the proximal stomach present already at birth, or an acquired metaplastic change of the distal esophagus due to gastric reflux, may postpone consensus regarding the organ from which tumors in that area originates. This is crucial considering that over the past 30 years, an increased incidence of adenocarcinomas of the distal esophagus and of the gastric *cardia* [43] has been registered in many Western countries. In 1993, Heidi *et al* [44] presented a detailed comparative study on the basis of 492 squamous carcinomas of the esophagus, 66 esophageal adenocarcinomas, 359 adenocarcinomas of the cardia, and 1288 infracardial gastric adenocarcinomas. They concluded that esophageal adenocarcinomas and cardiac adenocarcinomas should be considered as one single entity. Hence, according to these authors [44], the cardia belongs to the esophagus rather than to the stomach. Recent data from the Surveillance Epidemiology and End Results program [45] indicates that the incidence of esophageal adenocarcinoma among white American men increased 463%, from 1.01 per 100 000 person-years during

1975-1979 to 5.69 per 100 000 person-years during 2000-2004.

In the present survey it was found in fetuses that the squamous epithelium of the esophagus met directly with gastric pits without glands, whereas in two of the four stillborn baboons and in one 4 day old baboon, the squamous epithelium met directly the fundic mucosa. These results seem to be in conformity with the claims of Chandrasoma *et al* [14-18, 35-42], namely that at birth, the squamous epithelium of the esophagus meets directly with the oxyntic fundic mucosa of the stomach.

The first recordable size of the columnar-lined mucosa with mucous glands was registered in a 20 day old animal. Since all 4 animals of unknown age had recordable columnar-lined mucosa with mucous glands, it is conceivable that these 4 animals might have lived at least 20 days. The length of the CLMMG in the 38 post-natal NHPs varied considerably, from 0.8 mm to 25.2 mm. These variations were found to occur independently of the age or of the gender of the animals. The cause(s) for the considerable individual variations in the length of the CLMMG in NHPs remain unknown.

Compared to the human counterpart, the esophagus of NHPs often showed extensive columnar-lined metaplastic mucosa with mucous glands. One possible explanation for this finding could be that the regurgitated gastric secretion in NHPs is more acid than in the human counterpart [46] leading more frequently to CLMMG mucosa. However, Lakhoo *et al* [47] found that the pH of the gastric acid secretion in baboons is similar to that in humans.

Regurgitation has been for years considered a behavioural psychopathology in NHPs, being frequently compared to human disorders such as bulimia and rumination syndromes [27, 28, 47-52]. While a causal relationship between regurgitation and GERD has not yet been determined, a correlation between this behaviour and gastrointestinal disorders seems to exist [28]. At present it is assumed that regurgitation with rumination and consequently GER in NHPs is a natural physiological process [27, 28]. Regurgitation, rumination and consequently the reflux of gastric acid are often aggravated by heavy meals [27, 28]. Higher-ranking NHPs housed in a group setting usually have a greater access to food than lower-ranking NHPs [28]. This behaviour has been observed in numerous captive species of NHPs [47-52]. Consequently, despite that the pH of the gastric acid is similar in humans and in NHP at the time of pH testing, the reflux of that acid into the esophagus might have been more continuous in NHPs than in humans. It is to be understood that the NHPs received no medication for their naturally occurring rumination (with resulting GER), a treatment often instituted to humans with remittent waves of symptomatic GER. The possibility that the distal esophagus of NHPs is more sensitive to the acid regurgitation than the distal esophagus of humans could not be assessed in this investigation.

In the light of the aforementioned considerations, several questions arise. *i)* Does the high fatty diet and large meal diet together with regurgitation and re-ingestion of meals initiate and maintain columnar-lined esophagus in NHPs? In this context, it is today recognized that a high fat intake in humans relaxes the LES [53, 54], thus encouraging GER. This situation seems not to apply to NHPs inasmuch as the regular daily diet in monkeys includes only 4% fat, whereas the limiting dietary fat recommended by The American Heart Association in humans is 30% of total calories. Hence, the daily fat intake does not seem to be a factor that explains the high frequency of columnar-lined esophagus in NHP. *ii)* Does the position adopted during the gastric phase of the digestion play a role in the development of columnar-lined esophagus? In this respect, humans usually have an upright (orthostatic) position while NHPs adopt by nature, an oblique position during the gastric phase of digestion. This oblique position might have exacerbated the gastric reflux in NHPs. *iii)* Does the stress to which low-ranking NHPs are subjected to, encourage the development of columnar-lined esophagus? It is well known that stress both causes and increases the severity of symptoms in the gastrointestinal system [55-58]. Corticotropin-releasing factor (CRF) is the prime mediator of the stress response [59]. One response of CRF receptors to stress is an impaired or delayed emptying of the stomach contents, a situation that may encourage the development of GER. Recent research on the relationship of stress and heartburn suggests that a subset of individuals with GERD may be psychologically distressed

[57, 58]. It is well known that stress exposure [55, 58] may also lead to changes in the motility and in the function of the LES. As low-ranking NHPs housed in a group setting usually have a much lower access to food than high-ranking NHPs [40], it is not unconceivable that low-ranking NHP might have been subjected to daily stress. *iv)* Is the chemical component of the refluxate bathing the mucosal microenvironment responsible for the development of columnar-lined esophagus? Recently Mahadeva *et al* [60] found that GERD symptoms were more common and correlated with an increased endoscopic finding of columnar-lined esophagus in British patients than in South-East Asian patients. It was suggested that the different mucosal microenvironment rather than racial cause(s) were responsible for the differences in frequency of the columnar-lined esophagus in the two populations [60]. Consequently, possible differences in the chemical components of the refluxate bathing the esophageal mucosa microenvironment in humans and NHPs may help to explain the differences in the frequency and length of the CLMMG mucosa between the two species. *v)* Why a substantially extended columnar-lined mucosa in NHP failed to develop intestinal metaplasia (IM)? In humans, the presence of goblet cells is a criterion used to define the Barrett´s esophagus [61]. The goblet cells in the Barrett´s esophagus evolve through two aberrant genetic signals that orchestrate the differentiation in the intestine and colon, namely Cdx1 and Cdx2 [62]. These genetic signals are expressed even in the short Barrett´s esophagus, but not in the normal esophagus or in the stomach [35, 61]. Paradoxically, none of the NHPs here investigated showed goblet cell differentiation or other histological attributes of IM such as absorptive enterocytes with brush border (type I IM) or columnar cells with abundant cytoplasmic droplets (type II IM) and Paneth cell metaplasia [63]. Although the causes for this negative finding remain unclear, it might be possible that the genetic signals Cdx1 and Cdx2 acting in humans are not being activated in NHPs.

One possibility to explore the effect of one or more of the aforementioned factors on the mucosal phenotypes developing in the distal esophagus would be to assess the frequency of columnar-lined esophagus in NHPs at other facilities engaged in primate research. Different food regimens and behavioural attitudes at other facilities would identify whether these parameters have any bearing in the triggering of the histological changes compatible with columnar-lined esophagus in NHPs. Chandrasoma *et al* [35] reported that the length of the CM is superior to define severity of reflux disease in humans. Whether the length of the columnar-lined esophagus in our animals mirrors the severity of reflux disease could not be assessed in this study.

In conclusion, the present investigation substantiates the notion that the columnar-lined mucosa with mucus-producing glands is a post-natal developmental process in NHPs. These animals seem to offer an excellent spontaneous model to study the series of histological events that take place in the distal esophagus of NHPs, most likely under the influence of protracted GER.

Please address all correspondences to Carlos A. Rubio, MD, PhD, Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden. Fax: +46 8 51774524; Email: Carlos.Rubio@ki.se

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