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The Variation of the Fetal Esophageal Mucosa at the End of Gestation may be a Possible Etiopathogenic Factor for Barrett's Esophagus

La Variación de la Mucosa Esofágica Identificada al Final de la Gestación Puede ser un Posible Factor Etiopatogénico para el Esófago de Barrett

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SUMMARY: Barrett's esophagus is a condition where the distal third of the esophagus changes its epithelial lining from non-keratinized stratified squamous to simple columnar. This cross-sectional descriptive study was conducted to characterize the esophageal mucosa in the third trimester of pregnancy and determine possible variants in its development and was carried out in the Morphology Laboratory of the Health Faculty of the Industrial University of Santander, Colombia, with 45 human fetuses in the third trimester of gestation (weeks 25-40). A section of the distal esophagus and the first portion of the cardial region of the stomach were obtained, and the histological sections were subjected to a fixation process with 5 % formaldehyde solution. The sections were stained with hematoxylin and eosin and were evaluated for the presence of epithelial change or glands in the esophageal lamina propria. The change from non-keratinized stratified squamous epithelium to simple columnar epithelium was observed in the esophageal mucosa in five fetuses (11.1 %). In 15 cases (33.3 %), the presence of mucous glands underlying the epithelium was determined. In two fetuses, simple columnar epithelium was observed in the esophageal mucosa and underlying submucosal glands (4.4 %). The lack of replacement of the columnar epithelium by squamous epithelium in the distal third of the esophagus and the presence of mucous glands in the last third of gestation may suggest the presentation of Barret's esophagus in adulthood and thus, a predisposition to develop esophageal adenocarcinoma.

KEY WORDS: Barrett's esophagus; Adenocarcinoma; Fetuses; Epithelium.

INTRODUCTION

In mouse embryo models, the lumen of the esophageal mucosa has been found to be lined in its early stages by ciliated simple columnar epithelium, which is replaced by stratified squamous epithelium prenatally (Daniely *et al*., 2004; Yu *et al*., 2005; Que *et al*., 2007). The epithelial basal cells are responsible for the continuous renewal of the other cell types, which is maintained during postnatal life, maintaining homeostasis and epithelial repair in cases of injury (Jovov *et al*., 2011; Kalabis *et al*., 2012; Zhang *et al*., 2017).

As it occurs in mice, in human fetuses it has been postulated that, as has been described in mice, progressively throughout fetal life, in the process of esophageal histogenesis, the ciliated columnar epithelium is replaced. By the end of the third trimester of gestation, the mucosa the distal third of the esophagus presents a stratified squamous epithelium. This epithelium differentiates itself at the level of the gastroesophageal junction from the columnar epithelium characteristic of the gastric mucosa. (Gemonov & Kolesnikov, 1990; Hadravská *et al*., 2004). Tertychnyi˘ *et al*. (2012) report islets or patches of columnar epithelium on the surface of the laminated squamous epithelium, which would indicate unequal development of the esophageal mucosa until birth.

In postnatal life, mainly in adults and, to a lesser extent, in adolescents and children, Barrett's esophagus (BE) has been described, as a pathological condition in which the distal third of the esophagus changes its epithelial lining from stratified squamous non-keratinized to simple columnar due to the metaplasia. It is diagnosed by endoscopy and confirmed histopathologically by biopsy at the gastroesophageal junction (Falk *et al*., 2011; Fitzgerald *et al*., 2014). Its progression is due to continuous exposure of the esophagus to gastroesophageal reflux (GER) (Hassall,

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1997; Ford *et al*., 2005; Anderson *et al*., 2007; Coleman *et al*., 2011; Fall *et al*., 2011; Winberg *et al*., 2012; Andrici *et al*., 2013).

The importance of BE lies in the fact that it has become a risk factor for the development of esophageal adenocarcinoma (EAC) (Coleman *et al*., 2011; Shaheen, 2014). Little is known about the pathophysiology of this disease in children, although in newborns GER is considered physiological due to the immaturity of the cardiac sphincter, a situation usually lasting a few weeks and without permanent adverse effects on the mucosa of the third lower esophagus. (Ronkainen *et al*., 2005). BE studies in infants are especially limited by the number of samples and methodological design (Jeurnink *et al*., 2011; Twohig-Bennett *et al*., 2021).

The presence of microvilli in mucinous cells in the stratified squamous epithelium of the esophagus has been described (Shields *et al*., 1993; Sawhney *et al*., 1996). Additionally, a greater number of goblet cells have also been observed in the BE than in the epithelium of normal intestine. Of normal intestine (McIntire *et al*., 2011).

A study in humans suggests that the basal cell population exhibits heterogeneous behavior (Seery & Watt, 2000). In different esophageal pathologies such as cancer, the proliferation-differentiation of the epithelium is interrupted, which can lead to an invasion of the submucosa by an aberrant proliferation of the epithelium that finally leads to distant metastasis (Sachdeva *et al*., 2021).

Due to an incidental finding when observing lacunae or patches of glands and columnar epithelium in a sample slide of the lower third of the fetal esophagus, this study aims to characterize the esophageal mucosa in the third trimester of pregnancy, and to determine the possible variants in its development.

MATERIAL AND METHOD

This cross-sectional descriptive study was conducted on 45 human fetuses from 25 to 40 weeks from the collection of the Morphology laboratory of the Faculty of Health of the Industrial University of Santander in Colombia. The fetuses were grouped according to their gestational age (25-30 weeks, 31-35 weeks, and 36-40 weeks). The study and its procedures were in accordance with the Scientific Research Ethics Committee of the Industrial University of Santander and complied with Resolution 008430 of 1993, Decree 2164 of 1992 and Law 10 of 1990 of the Colombian Ministry of Health and the principles of Helsinki Declaration.

Extraction of the thoracoabdominal block was performed with a longitudinal I-shaped incision. The tongue was pulled down, detaching it with the esophagus, larynx, and trachea. Dissection was performed to detach the cardiovascular block and the esophagus cutting through the esophageal hiatus of the diaphragm to the stomach, obtaining a block with the tongue, esophagus and stomach. A section of the distal esophagus and the first portion of the cardial region of the stomach were obtained. After collection, the histological sections were subjected to a fixation process with 5 % formaldehyde solution. Then, the sections were stained with hematoxylin and eosin and were evaluated for the presence of epithelial change, and glands in the esophageal lamina propria.

Statistical analysis. Descriptive statistics and graphs were performed using SPSS 20 software (SPSS, Chicago, IL, USA) and Microsoft Excel 2013. Descriptive statistics were calculated for each morphometric parameter and the Kolmogorov Smirnov normality test was performed to each sample. Statistical significance of $p < 0.05$ was considered. Categorical variables were expressed as percentage. In the comparison of dichotomous qualitative variables, such as the presence or absence of simple columnar epithelium and mucous glands in the lamina propria in the distal third of the esophagus and their behavior between age groups, the chi-square test was used.

RESULTS

Histological evaluation of the 45 cuts at the level of the gastroesophageal junction was performed verifying that all the layers of these organs were fully recognized (mucosa, submucosa, external muscle and serosa). The change from non-keratinized stratified squamous epithelium to simple columnar epithelium was observed in the esophageal mucosa in five fetuses (11.1 %). In some cases large areas of columnar epithelium immersed between the squamous epithelium, with transition from the esophageal epithelial cells to columnar cells were observed (Fig. 1a). In other cases small plaques of columnar epithelium between the esophageal lining and with a total development of the columnar cells were detected (Fig. 1b). Characteristically, the esophageal glands are in the submucosa layer, which was verified in this study, but the presence of mucous glands underlying the epithelium was also identified in 15 specimens (33.3 %) (Fig. 2). In two fetuses, the presence of simple columnar epithelium was observed in the esophageal mucosa and, also, underlying submucosal glands (4.4 %) (Fig. 3). These glands, located under the esophageal epithelium presented mucosal-type cells, as occurs in the submucosal glands in each of the cases analyzed. A statistical relationship was found between the presence of mucous esophageal glands and the appearance of simple columnar epithelium in the esophageal mucosa ($p<0.001$).

Acoording to the gestational age group, simple columnar epithelium in the mucosa of the distal third of the esophagus was found in two cases in fetuses with a gestational age between 25-30 weeks, in one case between 31-35 weeks and in two samples between 36-40 weeks. Statistically significant differences among the different gestational age groups regarding the presence of simple columnar epithelium in the distal esophageal mucosa were not found (p=0.741).

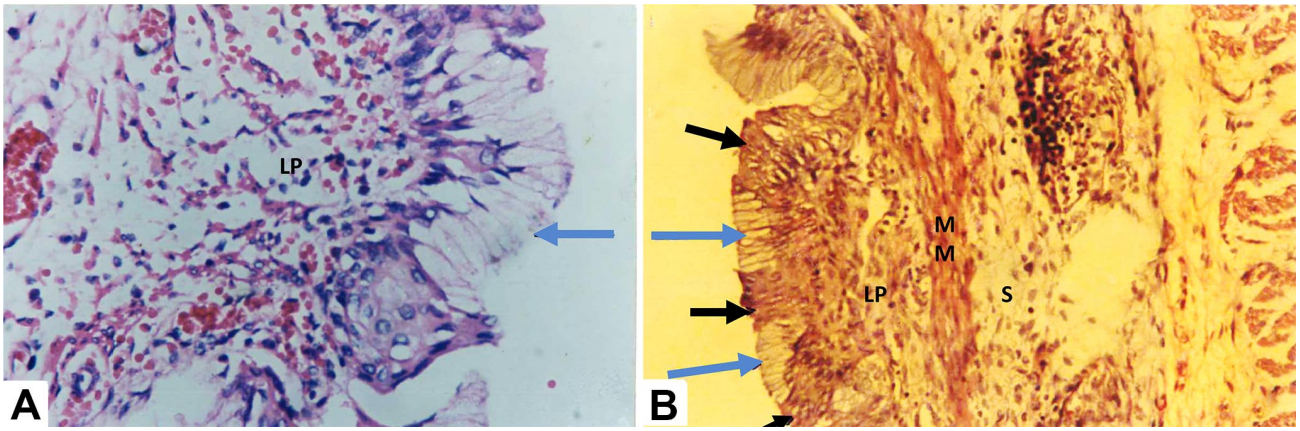

Fig. 1. Histological sections of the lower third of the esophagus. a. Presence of simple columnar epithelium (blue arrow) between plates of stratified squamous epithelium in a 26-week fetus. 40X. b. Presence of simple columnar epithelium (blue arrows) between plates of stratified squamous epithelium (black arrows) in a 29-week fetus. 10X. LP: lamina propria; MM: muscularis mucosa; S: submucosa.

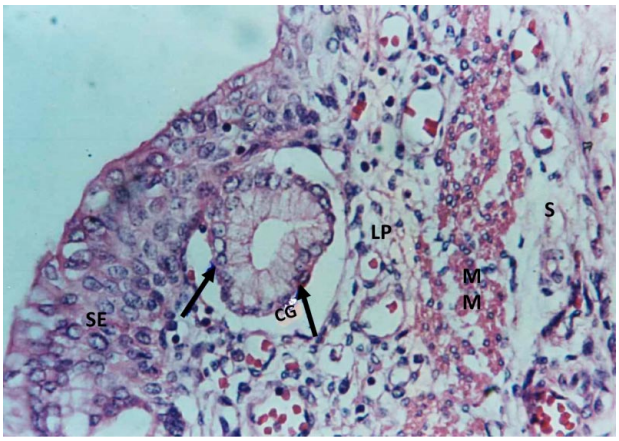

Fig. 2. Presence of cardial-type mucous gland (CG) in the lamina propria (LP) of the esophageal mucosa in a 30-week fetus. 40X. SE: squamous epithelium; MM: muscularis mucosa; S: submucosa.

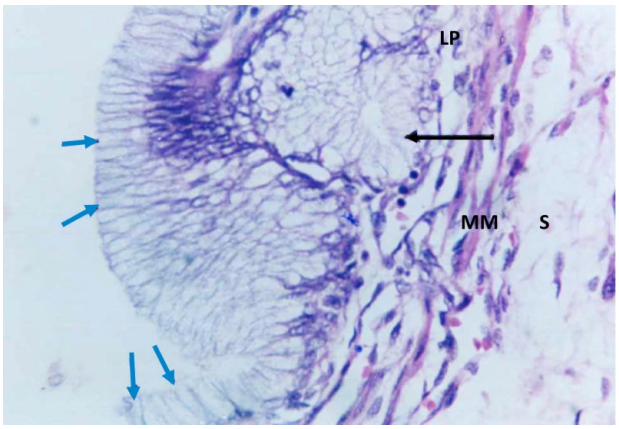

Fig. 3. Histological sections of the lower third of the esophagus in a 39-week fetus. Note the columnar epithelium in the lower third of the esophageal mucosa (blue arrows) and the presence of cardiac-type mucous glands in the lamina propria (LP). MM: muscularis mucosa; S: submucosa.

Regarding the mucous esophageal glands, they were found in five cases in each of the age groups studied. The presence of mucous esophageal glands did not present significant differences between the different age groups (p=0.873).

Finally, another incidental finding was the presence of ciliated columnar cells with eosinophilic cytoplasm, which was observed only in the three 27-week fetuses (Fig. 4).

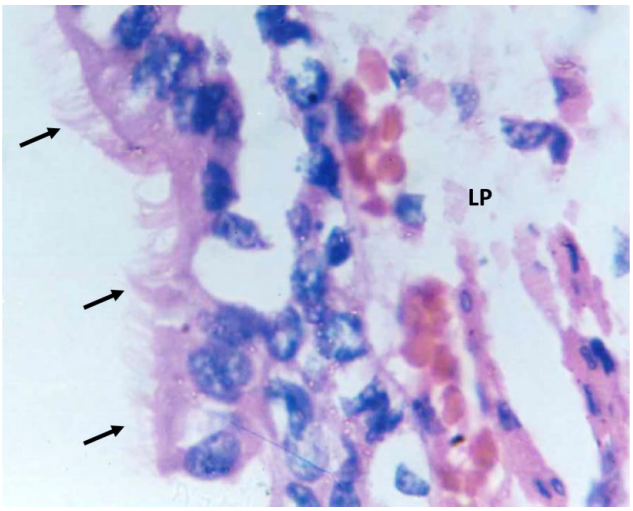

Fig. 4. Ciliated columnar cells with eosinophilic cytoplasm (arrows) in the lower third of the esophagus in a 27-week gestational fetus. 40X. LP: lamina propria.

DISCUSSION

Throughout prenatal life, the gradual replacement in the esophageal mucosa from a simple columnar epithelium to a stratified non-keratinized squamous epithelium has been reported in both mouse and human embryonic models (Gemonov & Kolesnikov, 1990; Hadravská *et al*., 2004; Yu *et al*., 2005; Que *et al*., 2007). Unlike what has been stated in the literature, there is evidence to suggest that the esophagogastric transition mucosa has gastric origin and arises before birth, presenting islets of columnar epithelium on the surface of the laminated squamous epithelium, which indicates its uneven development until birth. Thus, sites of immature epithelium could be considered as transformation zones of both laminated pavement epithelium and columnar epithelium (Tertychnyi˘ *et al*., 2012).

In this study, just as reported by Tertychnyi *et al*. (2012), we identified the persistence of this columnar epithelium in the form of patches or fragments immersed within the squamous epithelial tissue in 11 % of the esophagus evaluated; a histological characteristic of BE described since childhood and associated to the prolonged presence of GER. On their studies have noted a possible genetic predisposition or congenital etiology for BE, which has not been properly argued (Fahmy & King, 1993; Jeurnink *et al*., 2011; Sanchís *et al*., 2013).

Additionally, it has been described that the BE formation can also arise from aberrant gland duct epithelial cells located beneath the epithelium (Glickman *et al*., 2001; Nicholson *et al*., 2012). This type of glands was observed in our study in 33 % of the specimens suggesting that this histological pattern may persist in postnatal life and consolidate in individuals who present GER.

The time of exposure to GER without treatment is related to the development of BE in childhood. The prevalence of BE in childhood is significantly lower than in the adult population (6-12 % of adults compared to 0.3-4.8 % of children). In addition to the relationship with the degree and duration of GER. The longer the mucosa is exposed to GER without treatment, and therefore to the effects of acid, the greater the probability of esophagitis and development of metaplasia (Jeurnink *et al*., 2011; Sanchís *et al*., 2013).

Although BE is considered a premalignant condition, the incidence of carcinoma in the pediatric population is low. Long-term follow-up with endoscopies and staged biopsies seems advisable when there is evidence of BE and malignant alterations, as in the children who have BE with specialized mucosa and goblet cells (Hassall *et al*., 1993; Cotton *et al*., 2022).

The presence of microvilli in mucinous cells of the stratified squamous epithelium of the esophagus in adults has been described (Shields *et al*., 1993; Sawhney *et al*., 1996), and the presence of goblet cells in high numbers when BE is diagnosed (McIntire *et al*., 2011). In our study we did not find the presence of these structures, but we did describe ciliated columnar cells with eosinophilic cytoplasm as an incidental finding in the 27-week fetuses, which suggests in these cases, the consolidation of the metaplastic epithelium in the distal segment of the esophagus, as a variant feature of the histogenesis process of that organ with possible clinical implications in postnatal life.

The incidence of EAC in adults has been reported at 3 to13 % of patients suffering from BE (Cancer Research UK, 2020). This figure should be considered when evaluating childhood GER because the patterns of epithelial metaplasia and presence of glands in the mucosa in the process of esophageal histogenesis identified both by Tertychnyi˘ *et al*. (2012), and this study, besides GER, suggest the possibility that some cases of BE have an evident congenital component that warrants a permanent evaluation of its evolution.

CONCLUSIONS

The lack of replacement of the columnar epithelium by squamous epithelium in the distal third of the esophagus and the presence of mucous glands in the last third of gestation may suggest the occurrence of BE in adulthood and a predisposition to develop EAC.

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RESUMEN: El esófago de Barrett es una afección en la que el tercio distal del esófago cambia su revestimiento epitelial de escamoso estratificado no queratinizado a columnar simple. Este estudio descriptivo de corte transversal tiene como objetivo caracterizar la mucosa esofágica en el tercer trimestre del embarazo y determinar posibles variantes en su desarrollo y se realizó en el laboratorio de Morfología de la Facultad de Salud de la Universidad Industrial de Santander-Colombia, con 45 fetos humanos en el tercer trimestre de gestación (semanas 25-40). Se obtuvo una sección del esófago distal y la primera porción de la región cardial del estómago y las secciones histológicas se sometieron a un proceso de fijación con solución de formaldehído al 5 %. Los cortes se tiñeron con hematoxilina y eosina y se evaluaron determinando la presencia de cambio epitelial y glándulas en la lámina propia del esófago. El cambio de epitelio escamoso estratificado no queratinizado a epitelio cilíndrico simple se observó en la mucosa esofágica en cinco fetos (11,1 %). En 15 casos (33,3 %) se determinó la presencia

de glándulas mucosas subyacentes al epitelio. En dos fetos se observó epitelio cilíndrico simple en la mucosa esofágica y glándulas submucosas subyacentes (4,4 %). La falta de reemplazo del epitelio cilíndrico por epitelio escamoso en el tercio distal del esófago y la presencia de glándulas mucosas en el último tercio de la gestación pueden sugerir la presentación de esófago de Barrett en la edad adulta y una predisposición a desarrollar adenocarcinoma de esófago.

PALABRAS CLAVE: Esófago de Barrett; Adenocarcinoma; Fetos; Epitelio.

REFERENCES

Anderson, L. A.; Watson, R. G. P.; Murphy, S. J.; Johnston, B. T.; Comber, H.; Guigan, J. M.; Reynolds, J. V. & Murray, L. J. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World. J. Gastroenterol., 13(10)*:146-51, 2007.

Andrici, J.; Cox, M. R. & Eslick, G. D. Cigarette smoking and the risk of Barrett's esophagus: a systematic review and meta-analysis. *J. Gastroenterol. Hepatol., 28(8)*:1258-73, 2013.

Cancer Research UK. *Oesophageal Cancer Statistics*. Web Site. Oxford, Cancer Research UK, 2020. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer

Coleman, H. G.; Bhat, S.; Murray, L. J.; McManus, D.; Gavin, A. T. & Johnston, B. T. Increasing incidence of Barrett's oesophagus: a populationbased study. *Eur. J. Epidemiol., 26(9)*:739-45, 2011.

Cotton, C. C.; Eluri, S. & Shaheen, N. J. Management of Dysplastic Barrett's Esophagus and Early Esophageal Adenocarcinoma. *Gastroenterol. Clin. North Am., 51(3)*:485-500, 2022.

Daniely, Y.; Liao, G.; Dixon, D.; Linnoila, R. I.; Lori, A.; Randell, S. H.; Oren, M. & Jetten, A. M. Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. *Am. J. Physiol. Cell Physiol., 287(1)*:C171-81, 2004.

Fahmy, N. & King, J. F. Barrett's esophagus: an acquired condition with genetic predisposition. *Am. J. Gastroenterol., 88(8)*:1262-65, 1993.

Falk, G. W.; Jacobson, B. C.; Riddell, R. H.; Rubenstein, J. H.; El-Zimaity, H.; Drewes, A. M.; Roark, K. S.; Sontag, S. J.; Schnell, T. G.; Leya, J.; *et al*. Barrett's esophagus: prevalence incidence and etiology-origins. *Ann. N. Y. Acad. Sci., 1232*:1-17, 2011.

Fitzgerald, R. C.; di Pietro, M.; Ragunath. K.; Ang, Y.; Kang, J. Y.; Watson, P.; Trudgill, N.; Patel, P.; Kaye, P. V.; Sanders, S.; *et al*. British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut, 63(1)*:7-42, 2014.

Ford, A. C.; Forman, D.; Reynolds, P. D.; Cooper, B. T. & Moayyedi, P. Ethnicity, gender, and socioeconomic status as risk factors for esophagitis and Barrett's esophagus. *Am. J. Epidemiol., 162(5)*:454-60, 2005.

Gemonov, V. V. & Kolesnikov, L. L. Development of oesophageal tissue structures in human embryogenesis. *Anat. Anz., 171(1)*:13-5, 1990.

Glickman, J. N.; Chen, Y. Y.; Wang, H. H.; Antonioli, D. A. & Odze, R. D. Phenotypic characteristics of a distinctive multilayered epithelium suggests that it is a precursor in the development of Barrett's esophagus. *Am. J. Surg. Pathol., 25(5)*:569-78, 2001.

Hadravská, S.; Chlumská, A.; Boudová, L.; Mukensnabl, P. & Sulc, M. The histological findings in the gastroesophageal junction of fetuses. *Cesk. Patol., 40(1)*:7-10, 2004.

Hassall, E. Columnar-lined esophagus in children. *Gastroenterol. Clin. North Am., 26(3)*:533-48, 1997.

Hassall, E.; Dimmick, J. E. & Magee, J. F. Adenocarcinoma in childhood Barrett's esophagus: case documentation and the need for surveillance in children. *Am. J. Gastroenterol., 88(2)*:282-8, 1993.

Jeurnink, S. M.; van Herwaarden-Lindeboom, M. Y.; Siersema, P. D.; Fischer, K.; Houwn, R. H. & van der Zee, D. C. Barrett's esophagus in children: Does it need more attention? *Dig. Liver Dis., 43(9)*:682-7, 2011.

Jovov, B.; Que, J.; Tobey, N. A.; Djukic, Z.; Hogan, B. L. & Orlando, R. C. Role of E-cadherin in the pathogenesis of gastroesophageal reflux disease. *Am. J. Gastroenterol., 106(6)*:1039-47, 2011.

Kalabis, J.; Wong, G. S.; Vega, M. E.; Natsuizaka, M.; Robertson, E. S.; Herlyn, M.; Nakagawa, H. & Rustgi, A. K. Isolation and characterization of mouse and human esophageal epithelial cells in 3D organotypic culture. *Nat. Protoc., 7(2)*:235-46, 2012.

McIntire, M. G.; Soucy, G.; Vaughan, T. L.; Shahsafaei, A. & Odze, R. D. MUC2 is a highly specific marker of goblet cell metaplasia in the distal esophagus and gastroesophageal junction. *Am. J. Surg. Pathol., 35(7)*:1007-13, 2011.

Nicholson, A. M.; Graham, T. A.; Simpson, A.; Humphries, A.; Burch, N.; Rodriguez-Justo, M.; Novelli, M.; Harrison, R.; Wright, N. A.; McDonald, S. A. C.; *et al*. Barrett's metaplasia glands are clonal, contain multiple stem cells and share a common squamous progenitor. *Gut, 61(10)*:1380-9, 2012.

Que, J.; Okubo, T.; Goldenring, J. R.; Nam, K. T.; Kurotani, R.; Morrisey, E. E.; Taranova, O.; Pevny, L. H. & Hogan, B. L. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. *Development, 134(13)*:2521-31, 2007.

Ronkainen, J.; Pertti, A.; Storskrubb. T.; Johansson, J. E.; Lind, T.; Bolling-Sternevald, E.; Vieth, M.; Stolte, M.; Talley, N. J. & Agréus, L. Prevalence of Barrett's esophagus in the general population: an endocscopic study. *Gastroenterology, 129(6)*:1825-31, 2005.

Sachdeva, U. M.; Shimonosono, M.; Flashner, S.; Cruz-Acuña R.; Gabre J. T. & Nakagawa, H. Understanding the cellular origin and progression of esophageal cancer using esophageal organoids. *Cancer Lett., 509*:39-52, 2021.

Sanchís, G.; Ibáñez, V.; Couselo, M.; Fonseca, R.; Vila, J. J. & García-Sala, C. Risk factors for Barrett's esophagus in children: case-control study. *Cir. Pediatr., 26(1)*:13-6, 2013.

Sawhney, R. A.; Shields, H. M.; Allan, C. H.; Boch, J. A.; Trier, J. S. & Antonioli, D. A. Morphological characterization of the squamocolumnar junction of the esophagus in patients with and without Barrett's epithelium. *Dig. Dis. Sci., 41(6)*:1088-98, 1996.

Seery, J. P. & Watt, F. M. Asymmetric stem-cell divisions define the architecture of human oesophageal epithelium. *Curr. Biol., 10(22)*:1447-50, 2000.

Shaheen, N. J. What is behind the remarkable increase in esophageal adenocarcinoma? *Am. J. Gastroenterol., 109(3)*:345-7, 2014.

Shields, H. M.; Zwas, F.; Antonioli, D.; Doos, W. G.; Kim, S. & Spechler, S. J. Detection by scanning electron microscopy of a distinctive esophageal surface cell at the junction of squamous and Barrett's epithelium. *Dig. Dis. Sci., 38(1)*:97-108, 1993.

Tertychnyi, A. S.; Zhakota, D. A.; Mamchenko, S. I.; Andreev, A. I. & Hertog, H. Morphological features of the esophagogastric transition zone at fetuses and newborns. *Arkh. Patol., 74(3)*:10-4, 2012.

Twohig-Bennett, L.; Coleman, H. G.; Cairnduff, V.; McManus, D. T.; Murray, L. J.; Gavin, A. T. & Johnston, B. T. Esophageal columnar metaplasia in childhood: A population-based case series analysis. *Dig. Dis. Sci., 66(7)*:2317-22, 2021.

Winberg, H.; Lindblad, M.; Lagergren, J. & Dahlstrand, H. Risk factors and chemoprevention in Barrett's esophagus an update. *Scand. J. Gastroenterol., 47(4)*:397-406, 2012.

Yu, W. Y.; Slack, J. M. & Tosh, D. Conversion of columnar to stratified squamous epithelium in the developing mouse oesophagus. *Dev. Biol., 284(1)*:157-70, 2005.

Zhang, Y.; Jiang, M.; Kim, E.; Lin, S.; Liu, K.; Lan, X. & Que, J. Development and stem cells of the esophagus. *Semin. Cell Dev. Biol., 66*:25-35, 2017.

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