Paneth Cells in Colonic Adenomas: Association with Higher Adenoma Burden*

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ABSTRACT

Introduction. The association of Paneth cells with colorectal neoplasms has been demonstrated in several studies and case reports. The frequency of Paneth cell-containing adenomas ranges from 0.2 to 39% in the various published studies. Although adenomas with Paneth cells have already been recognized before, there are no studies in the Philippines that have been done to evaluate their clinicopathologic features. This study was performed to evaluate the clinicopathologic features of Paneth cell-containing adenomas and their association with adenoma burden.

Methodology. A total of 326 colorectal adenomas from 133 patients diagnosed consecutively from April 2013 to June 2013 at St. Luke's Medical Center, Quezon City, Philippines, were reviewed. These were checked for the presence of Paneth cells within the adenomatous crypts. The differences in gender and location were analyzed using one tail z-test, while the association of Paneth-cell containing adenomas with adenoma burden was analyzed using univariate odds ratio at 95% confidence interval.

Results. The frequency of Paneth cell-containing adenomas in this study of 326 adenomas is 15% (50 of 326 adenomas). There was no statistical significance in the occurrence of the lesion between male and female patients (32% vs. 15%; p=0.2041). There was also no statistical difference in their occurrence in the proximal and distal colon (18% vs. 14%; p=0.1723). The odds of having multiple adenomas for patients with Paneth cell-containing adenomas are 3.16 times higher than those patients without Paneth cell-containing adenomas (15 patients with one adenoma, 23 patients with more than one adenoma; p=0.0037).

Conclusion. This study has demonstrated the increased odds of harboring multiple adenomas in patients with Paneth-cell containing adenomas. This may be attributed in part to the fact that there have been recent studies revolving around Paneth cells that have shown that an established pathway of colorectal tumorigenesis, the APC/Wnt/β-catenin pathway, regulates differentiation towards this cell lineage.

Key words : adenoma, Paneth cells, colon

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INTRODUCTION

It was in 1872 that the Paneth cell was first recognized by Schwalbe and further studied in detail by Paneth.[1] As part of the innate immune system, antimicrobial products, such as defensins, are elaborated by Paneth cells.[1] These substances are present in the small intestine, particularly α-defensins.[2] The normal distribution of these cells in the gastrointestinal tract is from the duodenum to the ileum.[2] They are primarily seen at the base of the crypts of Lieberkuhn.[2] As stated by Andreu and colleagues (2008), "Paneth cells are filled with large apically located granules and have ultrastructural hallmarks (an extensive endoplasmic reticular and well-developed golgi) of prototypical secretory cells."[3] Paneth cells also secrete other products apart from α-defensins. These include antimicrobial proteins and peptides lysozyme, Reg3 γ, and secretory phospholipase A_2.[2]

The intestinal epithelium is in a constant state of proliferation, thereby continuously producing cells of all lineages.[3] The cellular mechanisms that drive this process have been linked to several molecular pathways, among the most crucial of which is the Wnt/β-catenin signalling. Commonly associated with sporadic colorectal cancers, the driving mutations of this pathway primarily involve the inactivation of the APC gene.[3] The unregulated proliferation of progenitor cells in inactivating mutations of the APC gene is due to the cytoplasmic accumulation of β-catenin. This leads to the latter being translocated to the nucleus where transcription factors activate the target genes of the pathway. Recently, it has also been discovered that differentiation towards the Paneth cell lineage also requires activation through the Wnt/β-catenin signaling pathway.[3]

The association of Paneth cells with colorectal neoplasms has been demonstrated in several studies and case reports. Rubio et al. reported a case of a 61-year-old man who had an adenoma with high-grade dysplasia that showed predominantly Paneth cells in the lower half of the villi and clusters of Paneth cells in the villous structures.[4] Similarly, Szumilo et al. presented a case of a large polypoid and ulcerated tumor arising from the hepatic flexure of the colon in a 76-year-old man who presented with hypogastric pain, constipation alternating with diarrhea, distension and weight loss. The tumor was diagnosed as a moderately differentiated adenocarcinoma. Paneth cells were then incidentally identified as part of the tumor. However, the authors concluded that they could not ascertain the impact of these neoplastic Paneth cells on prognosis since there are only a few reported cases in the available literature.[5]

The frequency of Paneth cell-containing adenomas ranges from 0.2 to 39% in the various published studies.[1] Although adenomas with Paneth cells have already been recognized before, there are no studies in the Philippines to the author's knowledge that have been done to evaluate their clinicopathologic features. The aims of the current study include the following: (1) to determine the frequency of Paneth-cell containing adenomas diagnosed consecutively at the St. Luke's Medical Center, Quezon City, Philippines, from April 2013 to June 2013; (2) to determine if gender and age are associated with the development of Paneth-cell containing adenomas; (3) to determine if the proximal location of the adenoma is associated with the presence of Paneth cells; and (4) to determine if the risk of harboring synchronous colorectal adenomas is associated with the presence of Paneth-cell containing adenomas.

METHODOLOGY

Study Population and Pathologic Evaluation of Colorectal Adenomas

The study population consisted of 326 colorectal adenomas from 133 patients diagnosed consecutively during a three-month period (April 2013 to June 2013) in St. Luke's Medical Center, Quezon City, Philippines. The slides were stained with routine Hematoxylin and Eosin. Seventy-nine patients had 1 adenoma, while 54 patients had two or more adenomas. The adenomas were classified as either tubular, tubulovillous or villous, depending on the percentage of villous architecture (less than 25%, 25-75% and more than 75%, respectively). Seven adenomas were classified as sessile serrated adenomas and one was diagnosed as filliform serrated adenoma. The author reviewed the adenomatous tubules within these adenomas. These were then evaluated for the presence of Paneth cells. In routine hematoxylin and eosin staining, the Paneth cells were easily recognized by their cytoplasmic features, which contained large, eosinophilic granules. An adenoma that has a dysplastic Paneth cell within its crypts can be classified as a Paneth cell-containing adenoma.[1]

The gender and age of the patients, as well as the location of the adenomas, were obtained from histopathology reports. The number of adenomas for each patient was reviewed with the endoscopy reports whenever discrepancies were identified. One patient was not included in the study because the number of adenomas could not be ascertained even after reviewing both the histopathology and endoscopy reports. Two patients underwent colonoscopy with biopsy twice. Regarding the location of the adenoma, the splenic flexure was used as the indicator to differentiate the proximal from the distal lesions (proximal if above the splenic flexure; distal if below the splenic flexure).[1]

Since we were limited by the information given in the histopathology report, several parameters could not be assessed adequately and, thus, narrowed the scope of our study. The sizes of the adenomas were not considered because some specimens were received piecemeal and their endoscopy reports did not state their actual sizes. It was also not taken into regard whether the entire colon was inspected during the endoscopic procedure and whether all polyps seen during endoscopy were biopsied and sent to histopathology. The clinical history, including those with a history of colorectal adenocarcinoma, familial adenomatous polyposis, or inflammatory bowel diseases, was not taken into account. Since there were only a few cases that demonstrated high-grade dysplasia, we did not take this into consideration as well.

Statistical Analysis

The sample size was determined using Epi Info 6.04d software (CDC, Atlanta, GA) with 95% confidence interval, power of 80%, and odds ratio of 3.12 based on the study of Pai et al. The minimum number of samples was 114. Adenomas from male and female patients with and without Paneth cells were analyzed using one tail z-test. Similarly, the location of the adenoma was also compared using one tail z-test. To determine the association of Paneth-cell containing adenomas with tumor burden, univariate odds ratio at 95% confidence interval was used.

RESULTS

The study population consisted of 133 patients with 326 adenomas. There were 73 male patients and 60 female patients.

The frequency of Paneth cell-containing adenomas in the 326 adenomas reviewed is 15% (50 of 326 adenomas). Of the 73 male patients, 23 (32%) had Paneth cell-containing adenomas (Figure 1). On the other hand, 15 (25%) of 60 female patients presented with Paneth cell-containing adenomas (Figure 1). However, there is no significant difference in the occurrence of Paneth cell-containing adenomas between male and female patients ($p = 0.2041$).

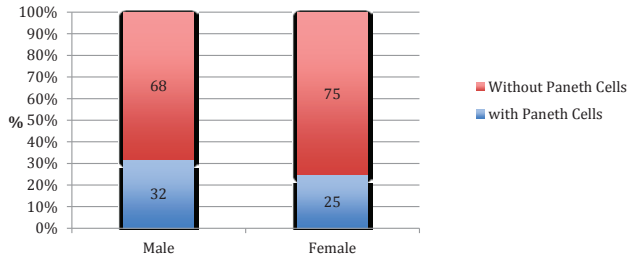

Figure 1. Distribution of subjects according to gender and presence of Paneth cells.

For patients with Paneth cell-containing adenomas, there is a sharp increase seen in patients aged 60-69 years. While for those patients without Paneth cell-containing adenomas, the peak is observed among patients aged 50-59 years.

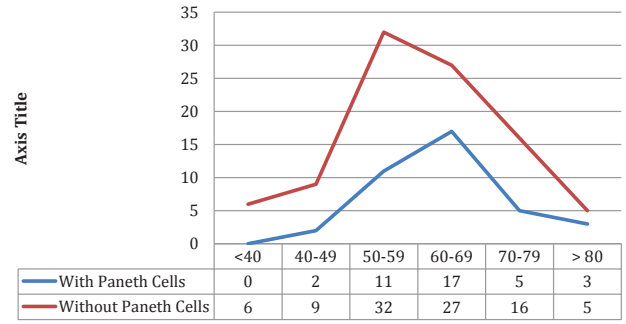

Figure 2. Frequency distribution of patients according to age group and presence of Paneth cells.

The Paneth cell-containing adenomas were also assessed according to their location. Most of the adenomas in this population were located at the distal colon (63% of adenomas).

Table 1. Distribution of adenomas per site

Site	No Paneth cell containing adenomas	With Paneth cell containing adenomas	Total number of adenomas per site	% Paneth cell-containing adenomas per site
Proximal	86	19	105	18%
Distal	178	29	207	14%
Unspecified	12	2	14	14%
Total	276	50	326	

Out of the 326 adenomas, 50 were classified as Paneth cell-containing adenomas. Thirty eight percent (38%) of the Paneth cell-containing adenomas were located at the proximal colon, while 58% of the Paneth cell-containing adenomas were located at the distal colon. However, looking at the proportion of Paneth cell-containing adenomas by location, 19 of the 105 adenomas (18%) in the proximal colon showed the presence of Paneth cells (Figure 3). In contrast, only 14% (29 of 207 adenomas) of the adenomas in the distal colon were classified as containing Paneth cells. Two of the Paneth cell-containing adenomas were excluded since they did not have the biopsy site specified. In spite of the increased proportion of Paneth cell-containing adenomas in the proximal colon, it was found that there was still no significant difference based on their site of occurrence (p = 0.1723).

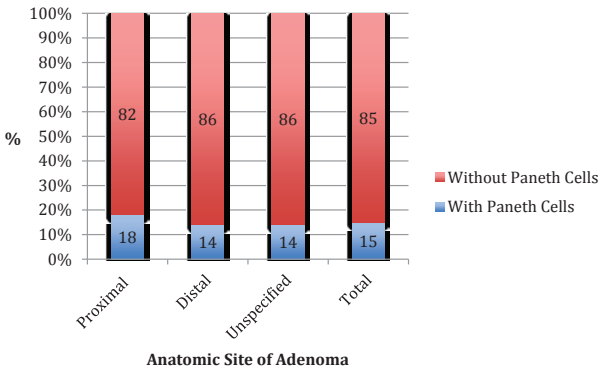

Figure 3. Proportion of Paneth cell-containing adenomas per site.

Among the 133 patients in the study population, 38 (29%) had Paneth cell-containing adenomas. Among these 38 patients, 15 had only one adenoma, while 23 had two or more adenomas (Table 2).

Table 2. Correlation of paneth cell-containing adenoma with adenoma burden

	Patients with More than One Adenoma	Patients with Only One Adenoma	Total
With Paneth-Cell containing Adenoma	23	15	38
Without Paneth-Cell containing Adenoma	31	64	95
% Paneth Cell-containing adenomas	43%	19%	
Total	54	79	133

Based on the above results, an odds ratio of 3.16 (confidence interval: 1.4-6.8) was computed. Thus, the odds of having multiple adenomas for patients with Paneth cell-containing adenomas are 3.16 times higher than those patients without Paneth cell-containing adenomas. Looking at the confidence interval, this is a significant finding (p = 0.0037).

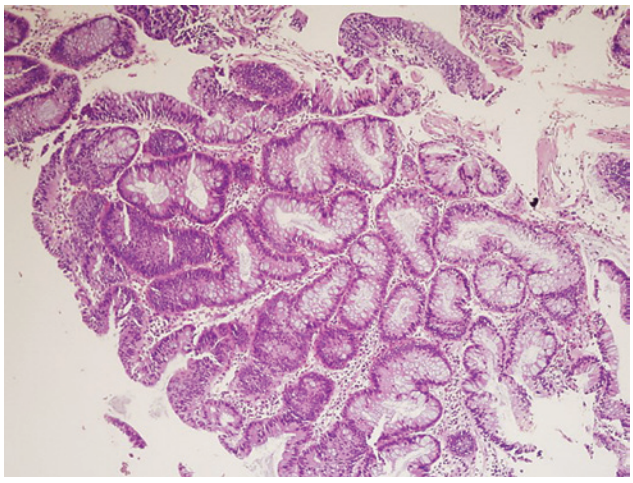

Figure 4. Tubular adenoma featuring at least low-grade dysplasia. The nuclei are pseudostratified, hyperchromatic and elongated. The architecture is predominantly tubular (40x, H&E).

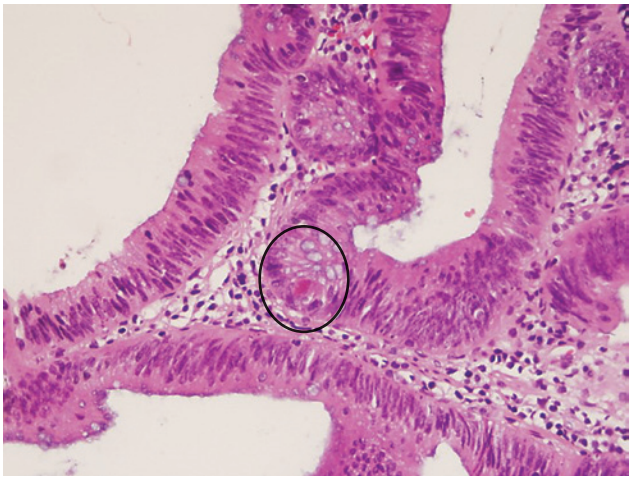

Figure 5. Paneth cell-containing adenoma was defined as the presence of a dysplastic Paneth cell(s) in the adenomatous crypts.[1] Occasionally, they can be found singly scattered along the length of the dysplastic glands (100x, H&E).

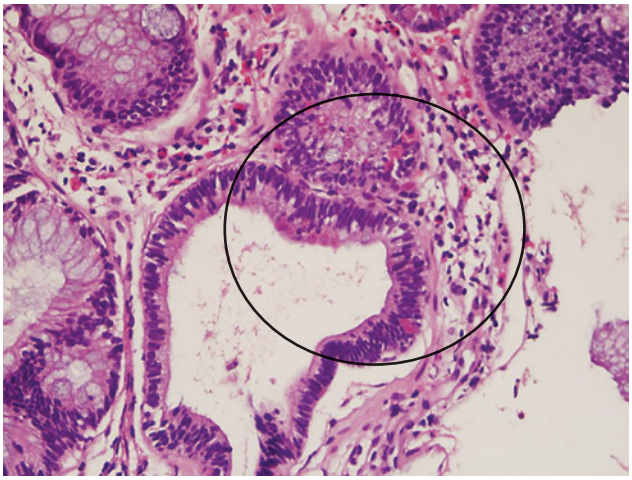

Figure 6. Paneth cells were also found clustered in small groups within the adenomatous crypts (100x, H&E).

DISCUSSION

Paneth cells can normally be found in the small bowel, appendix, and proximal colon. Here, they have an important role in innate intestinal immunity.[1] These cells express α-defensins, which, in the small intestine, aids in the elimination of Gram-positive and Gram-negative bacteria.[2] Apart from this function, Paneth cells have also been found to play a role in idiopathic inflammatory bowel diseases (IBD).[1] They serve as indicators of chronic injury in colitis, a role shared with mucous (pyloric) gland metaplasia.[8]

Epithelial neoplasms, such as adenomas, have been known to be occasionally associated with Paneth cell differentiation.[7] Four decades of case reports have demonstrated this association.[1]

The frequency of Paneth cell-containing adenomas in this study of 133 patients is 15%, which is consistent with the reported frequency of 0.2 to 39%. No significant difference was observed in the occurrence of Paneth cell-containing adenomas between male and female patients.

Although there is a peak in the occurrence of Paneth cell-containing adenomas among patients in their 50's and 60's, it is difficult to ascertain whether this finding is significant since, expectedly, most patients who undergo colonoscopy with biopsy belong to that age group. Perhaps a study that could obtain a population that is more evenly distributed among the different age groups would provide more comparable data regarding the association of increasing age with developing Paneth cell-containing adenomas. Pai et al. did not find any statistically significant association between age and the occurrence of these lesions.

It is not entirely surprising that the incidence of Paneth-cell containing adenomas is higher in the proximal colon than it is in the distal colon since Paneth cells are normal constituents of the former. Although there is an increased proportion of Paneth-cell containing adenomas among lesions of the proximal colon observed in this study, further analysis has shown that the difference in its occurrence in the proximal and distal colon was not statistically significant. These findings are in contrast to three other studies that have shown that Paneth cell-containing adenomas are more common in the proximal colon.[1,9,10]

Finally, analysis of the 133 patients in the study showed that the odds of having more than one adenoma for patients with Paneth cell-containing adenomas is higher than in those patients without Paneth cell-containing adenomas. In their study of 460 polyps from 200 patients, Pai et al. found that there is an increase in polyp burden in association with the presence of a Paneth cell-containing adenoma.[1] This may possibly be explained by recent investigations between the association of the APC/Wnt/β-catenin pathway and the differentiation and function of Paneth cells. Intestinal homeostasis and the maintenance of intestinal stem cells is controlled by the APC/Wnt/β-catenin pathway.[7] Furthermore, through the activation of this pathway, there is expansion of the crypt compartment. This is brought about by the stimulation of cell proliferation and the inhibition of cell migration and differentiation towards the enterocyte, goblet and enteroendocrine lineages. However, through its influence on transcription factors, the pathway also promotes differentiation towards Paneth cells.[7] This role has been shown in an experimental study on mice by Andreu et al. wherein they demonstrated that Paneth cell differentiation is also regulated by β-catenin signalling, apart from its well-known function in intestinal proliferation.[3] Consequently, the finding of Paneth cells in colorectal neoplasms may be linked to mutations in the APC/Wnt/β-catenin pathway since, in a similar fashion, the latter are also present in the formation of colorectal tumors.[7] Of note, none of the serrated colonic polyps (sessile serrated adenomas and filliform serrated adenoma) contained Paneth cells. These lesions harbor a different set of mutations (ex. microsatellite instability, DNA hypermethylation), which can be assumed to be part of the reason why they did not show any Paneth cell differentiation.

CONCLUSION

In summary, neoplasms of the colon, particularly adenomas, may harbor Paneth cells.[7] Although no statistically significant association with gender and site were observed in this paper regarding the presence of Paneth cell-containing adenomas, the trend favoring male patients and its occurrence in the proximal colon can already be seen, and perhaps, by increasing the population size, more significant results could have been obtained. Also, acquiring a population that is more evenly distributed among the different age groups would allow one to see if there is, in fact, an association with developing these lesions as age increases. More importantly, however, this study has demonstrated the increased odds of harboring multiple adenomas in patients with Paneth-cell containing adenomas. This may be attributed in part to the link between differentiation towards this cell lineage and the APC/Wnt/β-catenin pathway. As firmly established in scientific literature, the APC/Wnt/β-catenin pathway is responsible for the formation of a majority of colorectal tumors. However, since to the author's knowledge, there is only one other study that has delved into the association of Paneth cell-containing adenomas and tumor burden,[1] more research, preferably with a larger and more representative population, is still required to confirm these findings. Likewise, the significance of these findings and its impact on clinical practice, such as its possible effect on the interval of surveillance for colonoscopy, has yet to be determined.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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