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Serrated lesions of the appendix in serrated polyposis patients

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Summary

Patients with serrated polyposis develop multiple serrated polyps throughout the large bowel: hyperplastic polyps (HP), sessile serrated adenomas (SSA) and traditional serrated adenomas (TSA). The frequency and the characteristics of serrated lesions of the appendix have not been reported in serrated polyposis patients. We conducted a retrospective study of 34 serrated polyposis patients who underwent a total or right hemicolectomy for adenocarcinoma or polyp burden. An appendiceal serrated lesion was identified in 23 (68%): 13 SSAs, three SSAs with dysplasia, four HPs and three TSAs. The *BRAF*^{V600E} mutation was present in four polyps, all of SSA subtype (one with dysplasia). *KRAS* mutations were identified in 11 polyps (48%), in more than half of SSAs and of TSAs, and in none of the four HPs. None of the polyps displayed high levels of CpG island methylator phenotype (CIMP). There was no methylation in the promoter of the *MLH1*, *p16* or *MGMT* gene. Serrated lesions of the appendix are frequently found in serrated polyposis patients and are most commonly of SSA-type morphology, frequently associated with *KRAS* mutation. It is unclear if appendiceal serrated polyps are a feature of serrated polyposis or a lesion frequently identified in association with a proximal colonic adenocarcinoma.

Key words: Serrated polyps; serrated polyposis; appendix; KRAS; BRAF; CIMP.

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INTRODUCTION

Polyps in the large bowel displaying a serrated morphology are classified into three categories according to the World Health Organization (WHO): hyperplastic polyps (HPs) further divided into microvesicular and goblet cell HPs, sessile serrated adenomas (SSAs) also called sessile serrated polyps or sessile serrated lesions, and traditional serrated adenomas (TSAs).[1] Some of these polyps have the propensity to develop dysplasia and progress to malignancy through the serrated neoplasia pathway.[2] This molecular pathway to colorectal carcinoma (CRC) accounts for 15–30% of all CRCs and is characterised by the activation of the mitogen-activated protein kinase pathway through somatic mutations in the *BRAF* or the *KRAS* gene, and widespread DNA methylation, as defined by the CpG island methylator phenotype (CIMP). Precursor polyps to the serrated neoplasia pathway harbour some of these alterations, with varying proportions depending on the subtype of polyp and their location in the large bowel.[3] The *BRAF*^{V600E} mutation is present in 70–80% of microvesicular HPs, 80–90% of SSAs and 60% of traditional serrated adenomas TSAs.[4–7] Mutations in the *KRAS* gene are reported in 50% of goblet cell HPs, 20% of TSA, and extremely rarely in SSAs.[4,6,8] CIMP is frequently found in advanced SSA, in particular targeting the promoter of the *MLH1* gene and causing DNA mismatch repair deficiency.[5,9] In the appendix, polyps with a serrated morphology can be found. However, it has been debated whether the morphological criteria to diagnose serrated polyps should be used for the appendix, as the genotype-phenotype correlation seems to be different from what is reported in the rest of the large bowel.[10–12]

Serrated polyposis is a syndrome of unknown origin characterised by the occurrence of multiple serrated polyps in the large bowel and an increased risk of CRC for affected individuals and their relatives.[13–15] The definition of serrated polyposis is currently based on arbitrary clinical criteria and is likely to represent a heterogeneous group of individuals. The majority of patients with serrated polyposis have pan-colonic disease. In some patients, conventional adenomas are also present. The frequency and the characteristics of appendiceal serrated lesions in serrated polyposis patients have not been reported. In this study, we investigated the prevalence of serrated lesions in the appendix of serrated polyposis patients, classified the lesions according to the criteria for serrated polyps in the large bowel and performed molecular testing for *BRAF*^{V600E} and *KRAS* mutations and CIMP.

MATERIAL AND METHODS

Study samples

Cases were selected from Envoi Specialist Pathologists laboratory by searching the database using the key words 'serrated polyposis' and 'appendix', from January 2008 to December 2014. From all pathology reports retrieved, the following criteria were used for the final study group: patients had to meet the WHO criterion 1 and/or criterion 3 for serrated polyposis diagnosis[1] and the surgical specimen had to be a subtotal or right hemicolectomy with sampling of the appendix. Pathology and endoscopy reports were used for the number and the size of polyps. For criterion 1, at least five histologically confirmed serrated polyps proximal to the sigmoid colon, with two or more of these being >10 mm had to be diagnosed. For criterion 3, more

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than 20 serrated polyps of any size but distributed throughout the colon were required. Polyp number was cumulative over multiple endoscopic procedures. The WHO criterion 2 for serrated polyposis (any number of serrated polyps proximal to the sigmoid colon in an individual who has a first degree relative with serrated polyposis) was not used for patient selection, as information on family history was rarely given. Demographics data and other pathological findings were extracted from the pathology reports. This study was approved by the institutional review board of the QIMR Berghofer Medical Research Institute.

Histopathological review

The slides from each case with the sampled appendix, including a longitudinal section of the tip and two transverse sections, were reviewed by all three authors. The serrated lesions were classified into one of the serrated polyp subtypes using the 2010 WHO histological criteria for serrated polyp classification in the large bowel. Dysplastic serrated polyps included SSA with cytological dysplasia and TSA.

Molecular testing

DNA was extracted from the paraffin blocks using the Chelex-100 extraction method (Bio-Rad Laboratories, USA) after manual microdissection by a sterile scalpel blade to select the tissue area with the serrated lesion. The $BRAF^{V600E}$ mutation was detected by allelic discrimination as previously described[16] and *KRAS* mutation screening was performed by high-resolution melt analysis as previously described.[17] The CIMP was determined by the MethyLight technique with a panel of five markers (*CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1*).[18] High levels of CIMP (CIMP-high) was designated if three or more markers were methylated, low levels of CIMP (CIMP-low) if one or two markers were methylated and CIMP-negative when no marker was methylated. *MLH1*, *MGMT* and *p16* gene promoter methylation testing was performed as previously described.[4]

Statistical analysis

Statistical analyses were performed with SPSS statistics software version 17.0 (SPSS, USA). Comparisons for categorical variables were performed using Pearson's chi-squared test or Fisher's exact test where appropriate. A two-tailed p value was used for all analyses and values less than 0.05 were considered to be significant.

RESULTS

A total of 34 colectomy specimens with sampling of the appendix were identified in serrated polyposis patients. No macroscopic abnormality of the appendix was reported in any of the specimens. A serrated lesion was present in 23 specimens (68%). Patients' ages ranged from 29 to 90 years with a mean age at surgery of 71.1 years (Table 1). There was a slight female predominance. An adenocarcinoma of the colon was present in 74% of the resection specimens, the majority of them located in the proximal colon.

The appendiceal serrated lesions were classified into SSA for 13 (57%), SSA with cytological dysplasia for three (13%), microvesicular HP for four (17%), and TSA for three (13%) (Fig. 1 and 2, Table 2). All six dysplastic polyps of the appendix were found in patients with an adenocarcinoma: two in the caecum, two in the ascending colon, and two in the transverse colon. None of the patients with a surgical resection for polyps only had a dysplastic polyp in the appendix; five had an SSA and one had a HP. The association between dysplasia in appendiceal polyp and the presence of a colonic adenocarcinoma was not statistically significant ($p = 0.14$, Fisher exact test). The $BRAF^{V600E}$ mutation was present in four polyps, all of SSA subtype (one with cytological dysplasia). *KRAS* mutations were identified in 11 polyps (48%), in more than half of SSAs and of TSAs, and in none of the four HPs. None of the polyps displayed CIMP-high; CIMP-low was found in two lesions (two TSAs and one SSA). There was no methylation in the promoters of *MLH1*, *p16* or *MGMT*.

Table 1 Clinical characteristics of the 23 serrated polyposis patients with a serrated lesion of the appendix

Features	
Gender	13 females (57%)
Age at surgical resection	Mean 71.1 years (SD 15.1 years), range 29–90
Colectomy procedures	
(Extended) right hemicolectomy	16 (70%)
Subtotal	7 (30%)
Serrated polyposis criteria	
Criterion 1 only	14 (61%)
Criterion 3 ± criterion 1	9 (39%)
Adenocarcinoma	17 (74%)
Proximal adenocarcinoma location	15 (88%)
Cancer stages	
Stage I–II	11/17 (65%)
Stage III–IV	6/17 (35%)

SD, standard deviation.

DISCUSSION

Serrated lesions of the appendix have been previously known as mucosal hyperplasia or mucosal metaplasia.[19–21] After the description of the different subtypes of colorectal serrated polyps, studies have been performed to assess whether the terminology for colorectal serrated polyps could be used for appendiceal serrated lesions.[10–12] Bellizzi *et al.* categorised 37 serrated lesions of the appendix (from 53 non-invasive epithelial proliferations) into six HPs, 12 SSAs, three non-dysplastic HP/SSA lesions, and 16 mixed serrated and adenomatous lesions, possibly similar to some lesions that were classified as SSA with dysplasia or TSA in this study.[10] The authors reported an overlap in the morphology and the immunophenotype (CK20, Ki-67 and MUC6) between colorectal and appendiceal serrated lesions. More recently, Pai *et al.* evaluated the morphological features and the status of *BRAF* and *KRAS* genes in 46 serrated lesions from 132 appendiceal epithelial lesions.[11] None of these lesions were reported to be diagnosed in serrated polyposis patients. Interestingly, the authors reported a subcategorisation of appendiceal serrated lesions with similar proportions to our study when using the colorectal terminology: 7/46 versus 4/23 HPs, 21/46 versus 13/23 SSA, 9/46 versus 3/23 SSA with dysplasia, and 7/46 versus 3/23 TSA (two additional serrated lesions were classified as adenoma with serration in their series). Mutation rates in *BRAF* and *KRAS* were also comparable with the mutation rates of our study. We found *KRAS* mutation in 48% of all polyps (7/17, 54% of non-dysplastic polyps; 4/6, 67% of dysplastic polyps), compared with 52% (13/25, 52% of non-dysplastic polyps; 7/14, 50% of dysplastic polyps) in the Pai study. Our proportion of *BRAF* mutated polyps was slightly higher (22% versus 9%) and only identified in SSA with or without dysplasia. Based on the high *KRAS* mutation rate and the rarity of the $BRAF^{V600E}$ mutation, the authors suggested that the colorectal terminology should not be used for appendiceal serrated lesions and proposed that serrated polyps of the appendix should only be divided into dysplastic and non-dysplastic polyps.