RESEARCH ARTICLE

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Analysis of Immunohistochemical Expression of *BRAF* (V600E) Mutation in Serrated Colorectal Polyps: A Study from Tertiary Hospital in Oman

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Abstract

Background and aim: Colorectal cancer (CRC) is considered one of the most common cancers in the world. Serrated polyps were found to be precursor lesions for CRC. *BRAF* mutation (V600E) has been strongly linked to the development of these lesions. No previous study concerning *BRAF* immunohistochemical expression in serrated polyps- was done in Oman. The primary objective of our study was to assess the prevalence of *BRAF* (V600E) mutation in serrated colorectal polyps in the Omani population. The secondary objectives were to assess the prevalence of serrated polyps and their characteristic features: type, site and size as well as the relationship between *BRAF* (V600E) mutation and polyp type, site and size. **Materials and methods:** Ninety-one hyperplastic polyps (HP) (76.5%), 24 sessile serrated lesions (SSL) (20.2%) and 4 cases of tubular adenomas with low grade dysplasia (3.4%) were studied for *BRAF* (V600E) immunohistochemical expression. No case of traditional serrated adenoma (TSA) was present. Control cases of craniopharyngioma and papillary thyroid carcinoma were included. **Results:** *BRAF* (V600E) IHC was positive in 63 of the HP polyps (69.2%), 13 SSLs (54.2%) and none of the adenomatous polyps. The majority of positive polyps (75.0%) were ≤5 mm in size, 17.9% were 5-10 mm and 7.1% were ≥10 mm in size. The majority of *BRAF* (V600E) positive polyps (68.1 %) were in the distal colon and 31.9 % were in the proximal colon. The majority of positive cases for *BRAF* (V600E) were showing multiple polyps (61.8 %). None of the tubular adenomas showed any *BRAF* (V600E) positivity. **Conclusion:** Serrated polyps are now well known for their potential to develop CRC. Immunohistochemistry is an easy and reproducible way to detect *BRAF* (V600E) mutation. Our study showed there is high prevalence (64.3%) of *BRAF* mutation in serrated polyps in the Omani population. The majority of these polyps- were HP and SSL; and ≤5 mm in size and located in the distal colon.

Keywords: Colorectal cancer- colorectal polyp- serrated polyps- hyperplastic polyp- *BRAF* (V600E) mutation.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy in the world [1]. In Oman, it is considered the second most common cancer among Omani males and the third most common cancer among Omani females, according to Oman Cancer Registry 2016 [2].

The initiation and progression of CRC is driven by a stepwise accumulation of genetic alterations. There is however considerable genetic heterogeneity, and tumor subtypes that evolve through different pathways. In the "classic" progression model, inactivation of the adenomatous polyposis coli (APC) gene is an early initiating event, followed by additional alterations, such as KRAS and TP53 mutations. These classic tumors are more often located in the distal colon and rectum and genetically they frequently have chromosomal instability (CIN). In this adenoma-carcinoma sequence, adenomatous polyps are the neoplastic precursor lesions of adenocarcinoma [3].

Another type of colorectal polyps are the serrated polyps which are now believed to be precursor lesions to CRC through a distinct alternative pathway. Thirty years ago, these lesions were called "hyperplastic" polyps and were thought to have no malignant potential [4]. This concept was challenged by the observation of cancers developing in patients with hyperplastic polyposis syndrome or in sporadically occurring hyperplastic polyps [5]. Serrated polyps represent in fact several sub-entities, some of which are precancerous. The latest World Health Organization classification distinguishes three categories of serrated polyps: hyperplastic polyps (HPs), sessile serrated lesions (SSLs) with or without cytological

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dysplasia, and traditional serrated adenomas (TSAs) [6]. The major histologic feature of all these serrated polyps is the serrated in-folding (saw-toothing) of the crypt epithelium [7].

It has been estimated that up to 30% of CRC evolve from serrated polyps through a "serrated pathway". These CRC are considered to differ in their genetic characteristics from "classic" tumors arising through the adenoma-carcinoma sequence [4]. Serrated polyps predominantly have mutations in either *BRAF* or KRAS. They often exhibit high level microsatellite instability (MSI-H) and extensive DNA methylation of CpG islands, CpG island phenotype high (CIMP-H) [7]. *BRAF* mutation has been recognized in many types of cancer including malignant melanoma, hematolymphoid malignancies and some bone tumors [8-10].

After the first reports of *BRAF* mutations in colorectal malignancy by Davies et al., 2002, it has soon been recognized that *BRAF* alterations are strongly associated with right-sided serrated colonic cancer and its precursor serrated polyps [11]. The most frequent somatic alteration in *BRAF* is a point mutation (T1799A encoding BRAF (V600E), which results in increased activity of the protein's kinase domain. This causes sustained activation of the MEK1/2 → ERK1/2 mitogen-activated kinase (MAPK) signaling cascade- a pathway that controls a wide range of physiologic and tumor-promoting processes, including; self-renewal, proliferation, senescence, apoptosis, invasion, and metastasis- [11]. Experiments on mice showed that *BRAF* (V600E) is indeed the underlying initiating event that is sufficient for sustained hyperplasia induction. Later stages involve microsatellite instability, CpG island hypermethylation, WNT pathway activation and p16, p53 inactivation [7].

Systematic drug screening is promising for sensitivity of this CRC subtype to targeted therapeutics. Various MEK, PI3K, and *BRAF*-inhibitors- are in late-stage clinical development [11].

On review of literature, serrated colorectal polyps were studied in different Western and Asian populations. However, there is a lack of research in the Arab World except for one study in Saudi Arabia [12]. Over the studied five-year period (2004-2008), Bokhary studied a total of 325 colorectal polyps. Twenty-three of the originally diagnosed serrated polyps was found to have a true serrated morphology. The majority was found to be of the micro-vesicular subtype (12 cases = 52.2%). There were three goblet cell serrated polyps (13%) and two unclassifiable cases [12]. One study done in Iran in the year 2016 to investigate the frequency of serrated polyps/adenoma showed the following results: of 450 polyps, the frequencies of hyperplastic polyps, sessile serrated lesion (SSL) and traditional serrated adenoma (TSA) were 4.88%, 1.11% and 0.66% respectively while unclassified serrated polyps represented 0.44% [13].

A study on immunohistochemical expression profiles of *BRAF* (V600E/VE1) in serrated colon polyps in Turkish population, published in 2017, studied a total of 59 serrated polyps and showed the following: forty-one of 59 serrated lesions (69.4%) showed cytoplasmic VE1 staining, all SSL (100%); 92.8% of TSAs; 37% of HPs were stained positively [14].

In Oman, a retrospective study done in 2017, was conducted to describe the clinico-pathological characteristics of colonic polyps in Omani patients seen at Sultan Qaboos University Hospital. The study gave a general insight into the prevalence of different types of colonic polyps. The most common type of polyp was found to be adenomatous polyp in 88 (55%) cases, followed by serrated/hyperplastic polyp 51 (31.9%) [15]. However, this study was a general preliminary study. It did not study the prevalence of the different subtypes of serrated polyps or their malignant potential.

Hence, our study is the first of its kind in Oman. It was planned to investigate the prevalence of serrated colorectal polyps in Omani patients who presented to Sultan Qaboos Hospital, from the period of 2014 to 2019. It assessed the prevalence of *BRAF* (V600E) expression in these serrated polyps. It aimed to increase the awareness of both pathologists and gastroenterologists of these lesions. The results highlighted the importance of accurate detection and complete excision of these polyps.

Materials and Methods

This was a cross-sectional retrospective study that was conducted in Sultan Qaboos University Hospital (SQUH), Department of Pathology. It included biopsies of colorectal serrated polyps reported at SQUH during the period of 2014 to 2019.

Biopsies with diagnoses of hyperplastic polyp, sessile serrated polyp/adenoma/lesion or traditional serrated adenoma were included. These were retrieved from the SQUH Pathology Lab database (LabTrak). Ethical approval was obtained (MREC /SQU/239/17) and all Colonic polyps diagnosed as inflammatory or hamartomatous polyps were excluded. All demographic data of patients such as age, sex as well as presence or absence of multiple polyps- were obtained from the hospital information system (TrakCare). The following polyp characteristics were studied: polyp type, site and size. Polyp site and size were retrieved from both clinical details on specimen request form and from colonoscopy findings. Site of polyp was categorized as either proximal or distal. Proximal colon is defined as colon proximal to splenic flexure and distal colon was defined as distal to the splenic flexure. Polyp size was categorized into 3 main groups: ≤5 mm, 5-10 mm and ≥10 mm.

Total cases of 119 were studied. Haematoxylin and Eosin (H&E) slides of the cases were retrieved from the archive of the pathology department. These cases were reviewed by two pathologists with special interest in gastroenterology pathology, independently and each case was included in the study after consensus. Paraffin blocks were then retrieved and tissue microarray blocks were made with Beecher Manual Tissue Arrayer Model MTA-1. Duplicate punches were made for each case. Tissue microarray sections were then first stained with H &E. The *BRAF* (V600E) mutation was assessed by immune histochemistry (IHC) using recombinant anti-*BRAF* (mutated V600E) antibody [VE1] (ab228461) from Abcam, Cambridge, UK.

Tissue sections (5 µm) were mounted on amino-acetyl silane-coated glass slides (Starfrost, Berlin, Germany), sections were kept in hot oven over the night along with xylene for dewaxing, then descending grades of alcohol and distilled water for rehydration. Then, application of the primary antibody was preformed using the Utra system automated monostainer (Ventana Medical Systems) according to the manufacturer protocol. The antibody was diluted (1/100) and a heat mediated epitope retrieval with citrate buffer pH 6 was performed prior to commencing the IHC protocol. Positive staining of *BRAF* (V600E) was defined as non-equivocal cytoplasmic staining. Expression of *BRAF* (V600E) staining was categorized into 4 scores (Figure 1). Score 0 and 1 were defined as no and non-specific/equivocal staining, respectively. Scores 2 and 3 were defined as moderate and strong staining, respectively. Scores 0 and 1 were collectively reported as negative and scores 2 and 3 were reported as positive. Samples of caniopharyngioma and papillary thyroid carcinoma served as positive control and samples of tubular adenomas with low-grade dysplasia as a negative control, were included for comparison. The presence of *BRAF* (V600E) mutated proteins among serrated polyps was evaluated, and any correlation between *BRAF* (V600E) staining and polyp characteristics was also investigated.

Statistical Package for Social Sciences (SPSS) software, version 22, was used for statistical analysis. Continuous variables were summarized with mean ± standard deviation. Categorized variables were summarized by number and percentage. To assess relations between variables chi-square test was used. Qi square test was used for determining relationship between categorical variables. The Significance level was determined as $P<0.001$.

Results

A total of 119 polyps were included, 77% of whom were males and 42% of whom were females, with an age range of 17 to 81 years (mean = 55.18±14.13). Sixty-one percent of patients included in the study had multiple colonic polyps on colonoscopy studies (including serrated and adenomatous polyps); however, not all were included in our study, the cases which were included had multiple serrated polyps only.

There was 91 HP (76.5%), 24 SSL (20.2%) and 4 cases of conventional adenomas (3.4%). No case of TSA was present. All SSL except for a single case showed dysplastic changes. Fifty of included polyps (42%) had polyp size specified. The majority of these polyps were ≤5 mm (36 polyps, 72 %). Eleven polyps (22%) were 5-10 mm in size. Three polyps were 10 mm in size (6%). Size was not specified in 69 polyps (58%). Polyp site was mentioned in 110 cases (92.4%); 70 of these polyps (63.6%) were in the distal colon, while 40 polyps (36.4%) were in the proximal colon. Site was not mentioned in 9 cases (7.6%).

Seventy-six polyps (63.9%) were positive for *BRAF* (V600E) IHC. Of these, 55 (46.2%) showed moderate staining (score 3) and 21 (17.6%) showed strong staining (score 4). Forty-three polyps (36.1%) were negative for *BRAF* (V600E). Of these, 26 (21.8%) showed no staining (score 0) and 17 (14.3%) showed non-specific/equivocal staining (score 1).

BRAF (V600E) IHC was positive in 63 of the HP polyps (69.2%), 13 SSLs (54.2%) and none of adenomas. Correlation between type of polyp studied and *BRAF* (V600E) was statistically significant significant (p value

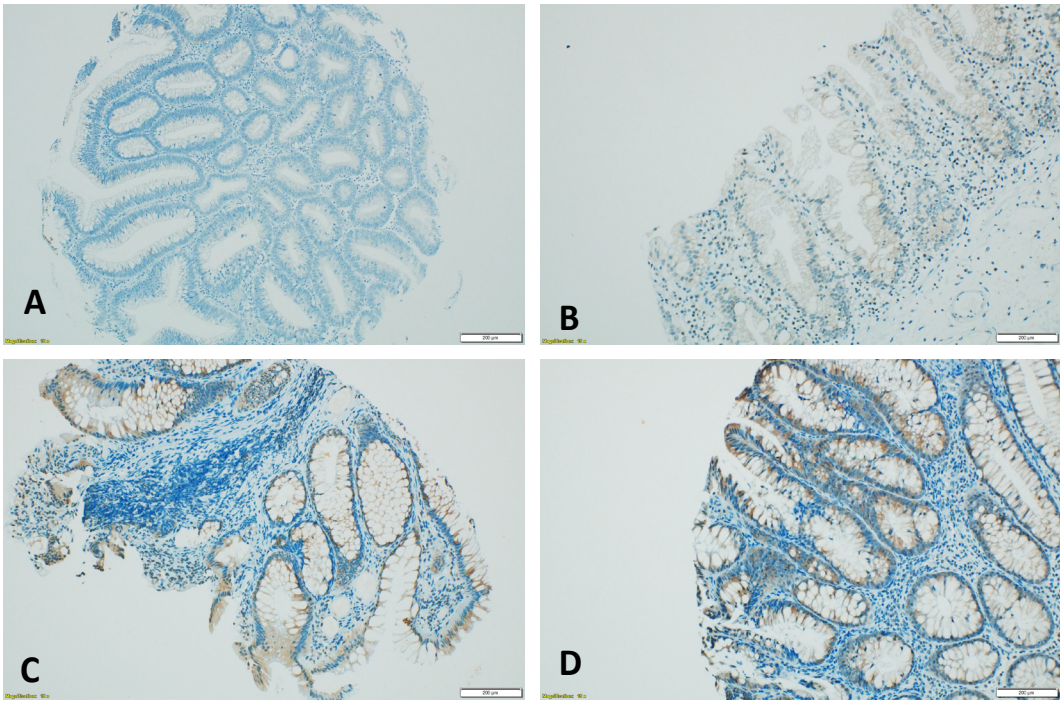

Figure 1. Immune Histochemical Staining of DifferentTypes of Polyp. Photomicrographs of tubular adenoma (A) with negative immune reactivity, hyperplastic polyp (B) with mild immune reactivity, sessile serrated lesion (C) with moderate immune reactivity and another sessile serrated lesion (D) with strong immune reactivity.

of 0.006). The majority of positive polyps (75.0%) were ≤5 mm in size, 17.9% were 5-10 mm and 7.1% were ≥10 mm in size. Relationship between size of polyp and *BRAF* (V600E) positivity was not statistically significant (p value of 0.700). The majority (68.1 %) of *BRAF* (V600E) positive polyps were in the distal colon and 31.9 % were in the proximal colon. Correlation study between polyp site and *BRAF* (V600E) was not statistically significant (p value of 0.207). The majority (61.8 %) of the positive cases for *BRAF* (V600E) showed multiple polyps. None of the tubular adenomas showed any *BRAF* (V600E) positivity.

The majority (82%) of size known HP were ≤5 mm in size and 64.2% of site-specified HP were located in the distal colon. Four (50%) of size-specified SSLs were ≤5 mm, 3 (37.5%) were 5-10 mm and 1 (12.5%) was ≥10 mm in size. The majority (14 polyps, 63%) of site-specified SSLs were located in the distal colon while 8 polyps (36.3%) were in the proximal colon. Polyp site was mentioned in 3 of the 4 included adenomas, 2 of these polyps were 5-10 mm and 1 was ≥10 mm in size. Two out of the tubular adenomas were in the proximal colon and 2 in the distal colon.

Discussion

This study investigated the immunohistochemical expression of *BRAF* (V600E) in serrated polyps. Serrated polyps are different from conventional adenomas not only by morphology but also by molecular characteristics. The most important molecular change in serrated polyps is the presence *BRAF* mutations. In this study, we tried to identify the frequency of *BRAF* mutation in serrated colorectal polyps with an immunohistochemical method.

In this study, we observed that 69.2% of HP, 54.2% of SSLs and none of the tubular adenomas were positive for *BRAF* (V600E) which constitutes 64.3% of serrated polyps. This reflects the high prevalence of *BRAF* mutation in serrated polyps. Within *BRAF* positive polyps, 82.9% were HP and 17.1% were SSL, which indicated that *BRAF* mutation was observed in HP polyps more frequent than SSLs.

This is in concordance with the Tuba et al. study (14) that showed a high frequency of *BRAF* staining in serrated lesions (69.4%). The majority of serrated polyps in their study were HP as in our study, however, *BRAF* mutation was found more frequently in SSL (100%) than HP (37%) while in our study it was more in HP. This could be due to the low sample number in their study (59 polyps) with only 18 SSLs.

The majority of positive polyps (84.2%) were ≤5 mm in size and 68.2 % were in the distal colon. However, statistically no significant relationship was traced between polyp site, size, type and *BRAF* expression, p value > 0.001. Compared with Tuba et. al study (14), our study found that the majority of positive polyps were in the distal colon which is discordant with their study- that found that majority of positive polyps were in the proximal colon. This could be explained that in our study polyps with the highest frequency of *BRAF* positivity were the HP polyps and these are usually located in the distal colon, while in the Tuba et. al study polyps with highest frequency for *BRAF* positivity were the SSLs which are usually located in the proximal colon.

Of note, serrated morphology is not a sufficient indicator for *BRAF* V600E mutation or a cancer risk alone. In our study we observed that 56% of HP and 62.5 % of SSLs were negative for *BRAF* expression. Hyperplastic polyps (HP) were the highest in prevalence, ≤ 5mm in size and located in majority in the distal colon in concordance with the findings of Bokhary et al. [12] and Mirzaie et al. [13] studies.

Some of the limitations in our study is the absence of TSA and the low number of conventional adenomas (4 polyps). Presence of TSA could have helped to gain some information about *BRAF* characteristics in these lesions as in other studies. The low number of conventional adenomas made it difficult to compare *BRAF* positivity between serrated lesions and adenomas. These limitations could be overcome by increasing the sample size in the future to include TSA and a comparable number of conventional adenomas; these were recommended to be correlated to the grade of dysplasia or follow up to progression to cancer. For better understanding of serrated polyps and their malignant potential, comprehensive prospective studies need to be carried out. Screening and surveillance methods for serrated lesions might consider testing for the presence or absence of *BRAF* V600E mutation.

In conclusion, serrated polyps are well known today to be responsible for the development of 30% of colorectal cancer through the serrated pathway. *BRAF* V600E mutation plays a major role in this pathway. *BRAF* V600E immunohistochemistry is considered a reproducible method that can be used to detect this mutation in serrated colorectal polyps. Within this study, we studied the prevalence of *BRAF* V600E in serrated polyps in a tertiary hospital in Oman and highlighted the significant high frequency of this mutation in our population. We hope that this work could help to increase the awareness of these lesions among pathologists and gastroenterologists and to lead to further studies for better characterization of these polyps in our population.

Author Contribution Statement

Conceptualization: Shalaby A, Qureshi A; Data curation: Shalaby A, Al Ghafri A; Formal analysis: Shalaby A, Al Ghafri A; Investigation: Sayed S, Al Badi S, Qureshi A; Methodology: Shalaby A, Al Husaini S, Sayed S; Project administration: Shalaby A; Resources: Shalaby A, Qureshi A; Supervision: Shalaby A; Writing - original draft: Al Ghafri A, Shalaby, Qureshi A; Writing - review & editing: All authors.

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Approval

The study was approved by the Ethical Committee at College of Medicine and Health Sciences Sultan Qaboos University Muscat, Oman with reference number: MREC SQU/239/17.

Availability of the data

All data are available upon request.

Conflict of interest

The authors disclose no conflict of interest.

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