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Original Article

Immunohistochemical expression profiles of BRAF (V600E/VE1) in serrated colon polyps in Turkish population

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Abstract: Background and aim: Colon carcinoma, as one of the most common cancers, has been investigated for genetic alterations. Besides well-known adenoma-carcinoma sequence, it is recently found that BRAF mutation had an important role particularly in early stages of adenocarcinomas with serrated features. There are no any studies concerning immunohistochemical expression status of BRAF V600E (VE1) antibody in serrated polyps in the Turkish population. The objective of this study is to observe the immunohistochemical staining of BRAF V600E (VE1) antibody in colon polyps in the Turkish population and investigate the frequency of presence of mutated BRAF proteins indicating malignant potential. Materials and methods: 59 cases of serrated polyps (27 cases of hyperplastic polyps, 18 cases of sessile serrated adenoma/polyps and 14 cases of traditional serrated adenomas) and 10 tubular adenomas, and 10 samples of normal colonic mucosa were immunohistochemically evaluated for the presence of BRAF V600E mutated proteins with the VE1 antibody. Results were statistically compared. Results: All SSA/Ps; 92.8% of TSAs; 37% of HPs were stained positively. Of the 27 hyperplastic polyps, all GCHPs were negative but 10 of 12 MVHPs (83.3%) were weakly positive with the VE1 antibody. Cases in control groups and tubular adenomas didn't show any cytoplasmic staining. Conclusion: Serrated adenoma/polyps have been gaining much more importance because of their malignant potential. Their frequency is also relatively high in the Turkish population and they should be carefully handled. Detection of BRAF V600E status can be easily achieved immunohistochemically by VE1 antibody. It is easily applicable and reproducible method and it might be helpful in identifying serrated lesions of the colon in addition to morphological features.

Keywords: BRAF immunohistochemistry, serrated polyps, VE1 antibody

Introduction

Colon cancer is the third most common malignant tumor in the world [1]. Many factors such as genetic, structural and environmental factors play significant roles in the development and spread of colon cancer. Chromosomal instability, microsatellite instability (MSI), aberrant DNA methylation, point mutations in oncogenes, and alterations in tumor suppressor genes are the most common genetic variations known so far [2].

Serrated pathway and BRAF mutation in colon carcinogenesis

The well-documented pathway in colon cancer development is occurred via so-called adenoma-carcinoma sequence by stepwise progression from adenomatous polyps to carcinoma. However, it is recently found that some carcinomas have been developed from serrated precursor lesions instead of adenomatous ones indicating a new pathway. Studies revealed that serrated types of colon adenocarcinomas originated from serrated polyps, constitute about 10-15% of colorectal cancers [3, 4]. In these cancers, BRAF mutation takes a crucial role in the early phase of carcinogenesis and this is an important component of the serrated pathway [5]. Therefore, serrated lesions with these mutations have a high risk of malignancy [5]. It has been demonstrated that BRAF gene was mutated in almost all sporadic colon carcinomas with microsatellite instability [6, 7]. On the

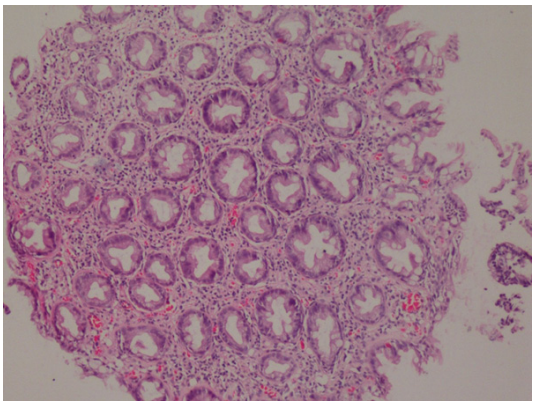

Figure 1. Hyperplastic polyp (microvesicular type) with prominent serrations. (H&E, ×20).

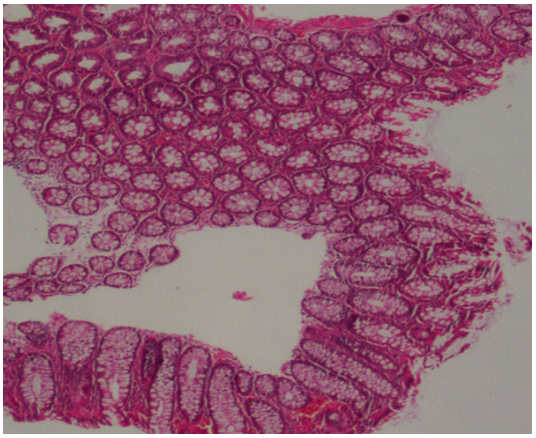

Figure 2. Hyperplastic polyp goblet cell rich type. (H&E, ×10).

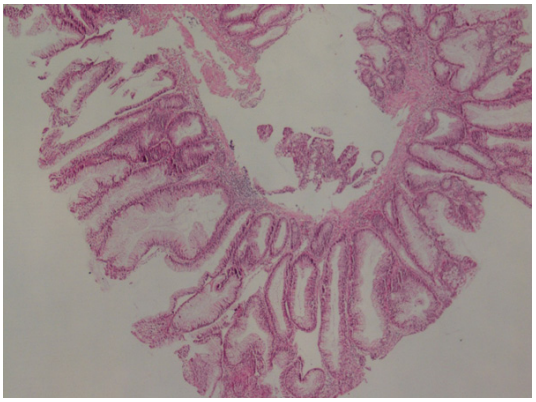

Figure 3. Serrated adenoma/polyp with dilated crypt base. (H&E, ×10).

other hand, it was reported that in cases with MSI lynch syndrome, BRAF gene mutation was almost unprecedented and also suggested to be used for excluding the Lynch syndrome [7-9]. It was also reported in many studies that the presence of BRAF mutation was related with poor prognosis and the response to treatment was poor in these cases [7, 10].

BRAF mutations can be detected by conventional PCR method, but this is not easy and applicable for every serrated lesion in routine practice. PCR method can also produce wrong negative results due to its insufficiency in determining the mutated cells in cases with a high number of normal mucosal cells [5]. Recently, detection of BRAF mutated proteins can be achieved by immunohistochemical methods and reliable results are obtained [6, 11-13].

Serrated polyps

Serrated polyps are frequently observed as a heterogenic group of lesions indicating a change with a serrated (saw tooth) shape in epithelial portion [14]. Characteristic features of a serrated polyp and its subtypes were designated in the literature [15, 16]. While the risk for cancer development from tubular/tubulovillous adenoma with dysplastic changes is foreseen as 35% in next 20 years, studies proposing any rate in serrated polyps are not completed yet [17].

Materials and methods

This study was approved by Institutional Review Board of Kayseri Research and Training Hospital (ID 150515/41-10).

In the present study, a total of 79 cases was selected from archives of Kayseri and Diskapi Research and Training Hospitals. In addition to 10 cases of normal colonic mucosa and 10 cases of tubular adenoma, and 59 colonic polyps with serrated morphology scanned and re-examined. According to the criteria of WHO, we re-classified the serrated polyps into three groups as hyperplastic polyp (n=27) (further subdivided into microvesicular (n=12) and goblet cell rich subtypes (n=15)), sessile serrated adenoma/polyp (SSA/P) (n=18), and traditional serrated adenoma (TSA) (n=14). We used briefly following criteria:

Microvesicular hyperplastic polyp (MVHP): They have prominent serrations in luminal parts of the crypts; straight crypts and narrow crypt

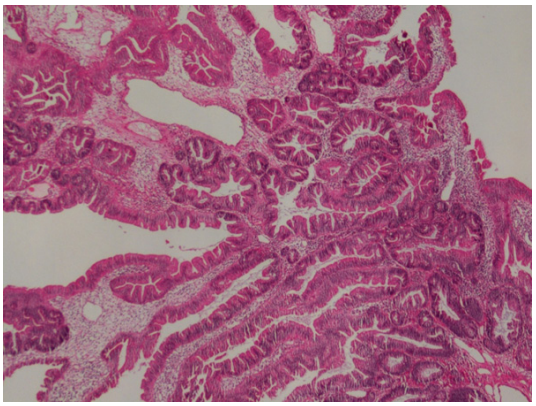

Figure 4. Traditional serrated adenoma with exophitic growth pattern and eosinophilic cytoplasm. (H&E, ×20).

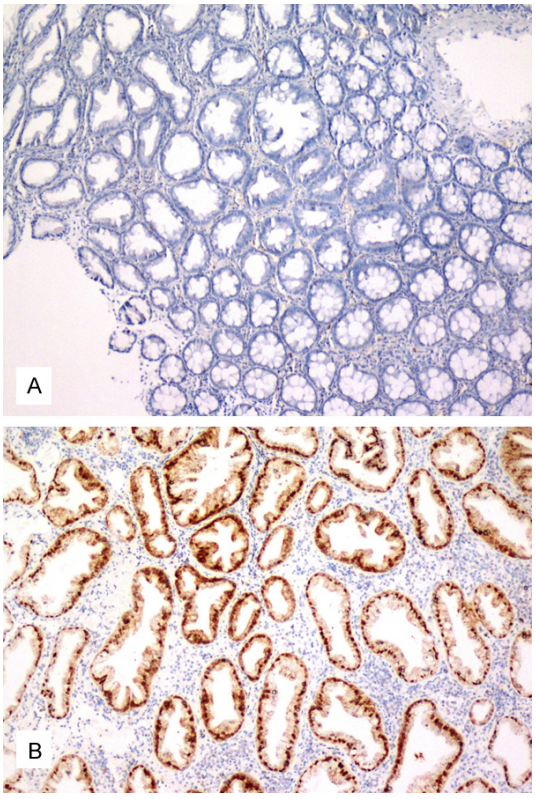

Figure 5. No staining (A) and nuclear staining (B) with the VE-1 antibody. (VE-1, ×10).

bases; and epithelial cells with small-droplet musin [5, 18] (**Figure 1**).

Goblet cell rich hyperplastic polyp (GCHP): They have straight crypts, fewer serrations and goblet-cell-rich epithelium [5, 18] (**Figure 2**).

Sessile serrated adenoma/polyp (SSA/P): They have dilatation and prominent serrations, abnormal crypt architecture (at least one L- or inverted T-shape crypt base) and asymmetrical proliferative zone [5, 15] (**Figure 3**).

Traditional serrated adenoma (TSA): They have villiform architecture, ectopic crypt formations, prominent serrations, eosinophilic cytoplasm and centrally located nuclei [5, 15] (**Figure 4**).

Following the required optimizations for staining, sections obtained from paraffin blocks of these cases were incubated at 70°C for one hour. Subsequently, stained with anti-BRAF/VE1 antibody (1:200 dilution) via the BenchMark XT automatic immune staining device using Opti-View Amplification. Stained slides were examined under a light microscope, evaluated by two pathologists independently (TDKU and AT) and each case was included the study after consensus. Mucin-poor types of hyperplastic polyps were not enrolled in the study because of a limited number of cases. The intensity of immunohistochemical staining was evaluated especially in serrated portions and regarded as negative or positive according to following criteria:

Negative: No staining (**Figure 5A**); Nuclear staining (**Figure 5B**).

Positive: Weak cytoplasmic staining (**Figure 6A**); Moderate cytoplasmic staining (**Figure 6B**); Strong cytoplasmic staining (**Figure 6C**).

The presence and frequency of BRAF V600E mutated proteins were evaluated and differences among study groups were noted. Any correlation between morphological features and staining characteristics is also investigated.

Statistical methods

Statistical analysis was performed by using the software IBM SPSS for Windows Version 22.0. Numerical variables were summarized with mean ± standard deviation and categorical variables were summarized by number and percentage. Kolmogorov Smirnov test was used whether the numerical variables exhibited normal distribution or not. Similarities of group variations were investigated by Levene test. If the hypothesis of parametric tests were pro-

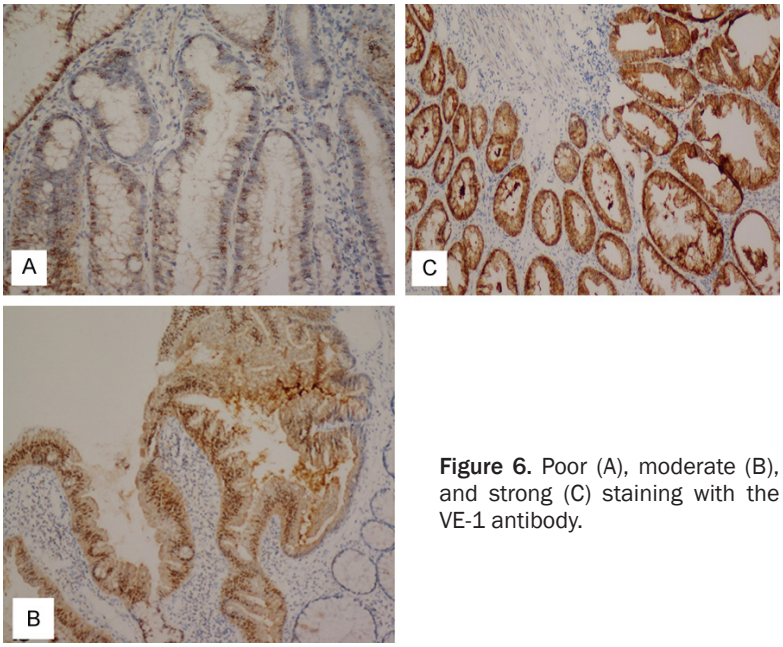

Figure 6. Poor (A), moderate (B), and strong (C) staining with the VE-1 antibody.

vided, t-test was researched in independent groups whether any difference is present between two groups in terms of numerical variables. Qi square test was used for determining the relationship between categorical variables. The Significance level was determined as P<0.001.

Results

A total of 59 serrated polyps (12 MVHPs; 15 GCHPs; 18 SSA/Ps and 14 TSAs), 10 tubular adenoma cases and 10 control cases were involved in this study. All patients were composed of 61 males (77.2%) and 18 females (22.7%) and mean age was 58.8±1.3 (range 15-84). Right colon was the most common location in MVHPs and SSA/Ps. Forty-one of 59 serrated lesions (69.4%) showed cytoplasmic VE1 staining. All SSA/Ps; 92.8% of TSAs; 37% of HPs were stained positively. Of the 27 hyperplastic polyps, all GCHPs were negative but 10 of 12 MVHPs (83.3%) were positive for the VE1 antibody. However, epithelial cells of SSA/Ps and TSAs showed stronger staining than those of MVHPs. All samples of normal colonic mucosa and conventional adenoma showed no staining. **Table 1** summarizes the subtypes of polyps, their anatomic locations and results of immunohistochemical staining with the VE1 antibody.

There was a statistically significant difference between SSA/Ps&TSAs and control cases and cases of conventional adenoma. (P<0.001) (**Table 2**). However, there was no significant difference between the traditional and sessile serrated subgroups (P= 0.445).

Also, any significant difference was not observed in serrated lesions considering the age of patients, location of the lesion, and size of the lesion.

Discussion

Our study indicated the high frequency of presence of BRAF mutated proteins in the SSA/Ps in the Turkish population.

The SSA/Ps have a main role in the currently called the serrated pathway of colorectal carcinogenesis. For long years, conventional adenomatous polyps have been known as only precursor lesions in the development of colon cancer and have been the main focus of interest for researchers, clinicians, and even pathologists. On the one hand conventional adenomas have been a mainstay of cancer screening programs, on the other hand many lesions showing specific serrated morphology have been generally diagnosed as hyperplastic polyp and considered as having no malignant potential. However, there has been increasing number of studies revealing that serrated polyps were not innocent in the development of colon carcinoma contrary to popular opinion [16, 19]. In addition to the presence of their malignant potential, studies revealed also that SSA/Ps have more aggressive behavior than conventional adenomas in cancer progression [20].

The SSA/Ps are different from conventional adenomas not only by morphology but also by molecular characteristics. The most important molecular change in SSA/Ps is considered BRAF mutations in epithelial cells [5]. In this study, we tried to identify this change with an immunohistochemical method and to evaluate the frequency of BRAF mutated proteins in the Turkish population.

In our study, we observed all SSA/Ps and most of the TAs are positively stained by the VE1 anti-

Table 1. General features of lesions ond results of immunohistochemical staining with VE1 antibody

Diagnosis	Localisation	BRAF VE1 IHK	Staining intensity		
			Strong	Moderate	Weak
Microvesicular hyperplastic polyp	All (n=12)	10/12 (83.3%)	0/10	0/10	12/10
	Right colon (n=6)	5/6 (83.3%)	0/5	0/5	5/5
	Left colon (n=3)	3/3 (100%)	0/3	0/3	3/3
	Transverse colon (n=3)	2/3 (66.6%)	0/2	0/2	0/0
Goblet-cell-rich hyperplastic polyp	All (n=15)	0/15 (0%)	0/0	0/0	0/0
	Right colon (n=1)	0/1 (0%)	0/0	0/0	0/0
	Left colon (n=11)	0/11 (0%)	0/0	0/0	0/0
	Transverse colon (n=3)	0/3 (0%)	0/0	0/0	0/0
Sessile Serrated adenoma/polyp	All (n=18)	18/18 (100%)	6/18	12/18	0/18
	Right colon (n=13)	13/13 (100%)	4/13	9/13	0/13
	Left colon (n=4)	4/4 (100%)	2/4	2/4	0/4
	Transverse colon (n=1)	1/1(100%)	0/1	1/1	0/1
Traditional serrated adenoma	All (n=14)	13/14 (92.8%)	2/13	8/13	3/13
	Right colon (n=3)	3/3 (100%)	0/3	3/3	0/3
	Left colon (n=10)	9/10 (90%)	2/9	4/9	3/9
	Transverse colon (n=1)	1/1 (100%)	0/1	1/1	0/1
Tubular adenoma	All (n=10)	0/10 (0%)	0/0	0/0	0/0
	Right colon (n=2)	0/2 (0%)	0/0	0/0	0/0
	Left colon (n=6)	0/6 (0%)	0/0	0/0	0/0
	Transverse colon (n=2)	0/2 (0%)	0/0	0/0	0/0
Control	All (n=10)	0/10 (0%)	0/0	0/0	0/0
	Right colon (n=1)	0/1 (0%)	0/0	0/0	0/0
	Left colon (n=8)	0/8 (0%)	0/0	0/0	0/0
	Transverse colon (n=1)	0/1 (0%)	0/0	0/0	
Total	79	41			

Table 2. Immunohistochemical BRAF (VE1) staining profile in study groups

		Hyperplastic polyps		*Serrated adenomas*				
		MVHP (n=12)	GCHP (n=15)	SSA/P (n=18)	TSA (n=14)	TA (n=10)	Control (n=10)	*P* Value*
Cytoplasmic staining with VE1 antibody	Negative	2 (16.7%)	15 (100%)	0	1 (7.2%)	10 (100%)	10 (100%)	< 0.001
	Positive	10 (83.3%)	0	18 (100%)	13 (92.8%)	0	0	

MVHP: Microvesicular hyperplastic polyp; GCHP: Goblet cell rich hyperplastic polyp; SSA/P: Sessile serrated adenoma/polyp; TSA: Traditional serrated adenoma; TA: Tubular adenoma; *Significant difference between serrated adenomas and other groups.

body which shows BRAF mutated proteins. There was a significant difference between SSA/Ps and other groups. Among HPs, the considerable amount of MVHPs showed also positive staining. But cases of SSA/Ps had stronger staining than cases of MVHPs as compatible with the literature [5]. This finding also supports the theory that the MVHPs are precursors and may progress into SSA/Ps [21].

We observed in some cases which have intense positive staining by the VE1 antibody, a sharp separation between serrated areas and neighboring normal glands. We think that this supports a clonal evolution of BRAF V600E mutation. In the literature, it was pointed out that the majority of adenocarcinomas with BRAF V600E mutations were mostly located in right colon [4]. Although the VE1 (+) SSA/Ps were mainly located in the right colon in our study, no difference was traced between right and left colon-located serrated polyps.

Our current findings obtained from this immunohistochemical study revealed the presence of BRAF V600E mutated proteins in most of the

sessile and traditional serrated polyp/adenomas. In accordance with the literature, this study indicated that the frequency of BRAF V600E mutant proteins in these lesions was significantly higher than those in hyperplastic polyps. Therefore they have more a pronounced risk for cancer development compared to hyperplastic polyps and they should be handled with a different protocol. Data related to the classification, recognition, and identification of serrated lesions was limited, but studies are in progress. Thus, standardized and validated surveillance guidelines for serrated polyps are not present yet [14, 18].

There are difficulties in assessment of serrated lesions in routine practice and there is a great interobserver variability even among expert pathologists [22]. We can say that BRAF VE1 antibody highlighted serrated nature of the lesion well. This immunohistochemical staining would also help us in the evaluation of biopsy samples even with orientation problem in routine practice and could contribute to an accurate diagnosis, especially for pathologists who do not have enough experience in this field.

On the other hand, we all know that serrated morphology is not a sufficient indicator for BRAF V600E mutation or a cancer risk alone, because we observed that only 37% of hyperplastic polyps-which also have serrated morphology-showed VE-1 positivity and none of TAs-which have malignant potential but not serrated morphology. However, all SSA/Ps-which have high risk of malignancy-showed moderate or strong positivity. We believe that in the development of a screening and surveillance method for serrated lesions it might be considered the presence or absence of BRAF V600E mutated proteins in addition to the morphology.

Although the presence of BRAF V600E mutation in serrated lesions is so high, the relatively low number of colon adenocarcinomas with serrated characteristics which show the presence of BRAF V600E mutated proteins was a remarkable situation. It is known that BRAF V600E mutation is an early step in the serrated pathway of carcinogenesis. Because of these reasons, there is no doubt that for a better understanding of the mechanisms of this process, comprehensive prospective studies need to be carried out.

Conclusion

In conclusion, immunohistochemical determination of proteins with BRAF V600E mutation is easy to apply and a reproducible method and it can be used in the evaluation of SSA/Ps. In this context in addition to other morphological criteria, we believed that BRAF V600E antibody could help both pathologists and clinicians and could be used as a guiding marker for overcoming serrated polyps. With this study, we want to underline the importance of serrated adenoma/polyps by indicating the presence of BRAF V600E mutant proteins in these lesions and their substantially high frequency. We hope that their identification and detection at early stages with accurate evaluation will lead a decline in colon cancer with serrated properties.

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Disclosure of conflict of interest

None.

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