

Detection of BRAF^{V600E} Biomarker in Patients with Colorectal Cancer Using Immunohistochemical Techniques / A Clinic-Pathological Study

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Abstract:

Background: Colorectal cancer (CRC) is the fourth common gastrointestinal cancers. (BRAF^{V600E}) is a member of RAF family of serine/threonine protein kinases that function to regulate the (MAPK) / (ERK) pathway. BRAF^{V600E} mutated CRC are associated with right-sided primary tumors, older women, high-grade tumors and poor prognosis

Objectives : Detection of BRAF^{V600E} mutation in a sample of Iraqi patients with CRC using immunohistochemical techniques and study the correlation of BRAF^{V600E} biomarker with other clinicopathological variables such as patient's age and tumor grade.

Materials and Methods : In this case series study a total of 90 colorectal cases of Iraqi patients were collected from teaching labs of Al-Yarmouk Teaching Hospital during the period of 20 September 2019 to 20 May 2020 including 60 cases of CRC and 30 cases of colorectal adenoma , The samples were studied regarding the expression of BRAF^{V600E} biomarker and its relationship to certain clinico-pathological parameters including : Patient's age, sex, tumor site, tumor size, histological type, pathological grade and pathological stage.

Results: Regarding the carcinoma group, BRAF^{V600E} biomarker was positive in 10 (16.6%) cases of CRC group and all adenoma cases showed negative expression of BRAF^{V600E}.

Conclusions: BRAF expression was higher in older patient's ages and higher tumor stages and there is no expression of BRAF^{V600E} in adenoma cases.

Keywords: CRC: Colorectal cancer, BRAF: v-raf murine sarcoma viral oncogene homolog B1, MAPK : Mitogen activated protein kinase.

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Introduction:

Colorectal cancer (CRC), also well-known as bowel cancer, is a cancerous growth affecting the colon, the rectum, or both.¹ The American Cancer Society estimates that around 1 in 21 men and 1 in 23 women in the United States will advance CRC during their life.^{1,2} Worldwide, colorectal carcinoma is the second most common malignancy and the fourth most common cause of cancer death after lung, stomach and liver cancers.¹ The developed world accounts for over 63% of all cases. It ranging from more than 40 per 100,000 people in the United States, Australia, New Zealand, and Western Europe to less than 5 per 100,000 in Africa and some parts of Asia.³ The BRAF serine threonine protein kinase is a downstream signaling protein in the epidermal growth factor receptor mediated mitogen activated protein kinase pathway, which motivates MEK through its phosphorylation. The mutation of codon 600 within the BRAF kinase domain leads to continuous activation of the MAPK pathway. In the early and late stages, nearly 8–14% of (CRC) express BRAF^{V600E} mutation.⁴⁻⁸

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BRAF^{V600E} mutated CRC are associated with right sided primary tumors, older women, high grade tumors, and precursor sessile serrated adenomas.⁹ They express a distinctive pattern of metastatic spread, with higher rates of peritoneal involvement, Distant lymph node metastases, and a lower frequency of lung metastases. 10, 11 just a while ago, the detection of BRAF mutations was done with PCR assays. These methods require a large number of malignant cells and extraction of the DNA. Immunohistochemical detection of BRAF with a mutation specific antibody was first described in metastatic melanoma and papillary thyroid carcinoma.¹² The convenience of immunohistochemistry (IHC) over PCR based assays is the smaller amount of the required tissue and the availability of this method in most pathology laboratories.^{12,13}

Cases and methods:

In this case series study, a total of 90 colorectal cases of bowel resection specimens of Iraqi patients were collected from the teaching labs of Al-Yarmouk Teaching Hospital during the period of 20 September 2019 to 20 May 2020 including

60 cases of carcinoma and 30 cases of adenoma. All the preparations for IHC were performed in Al-Yarmouk Teaching Hospital, Baghdad. The staining procedures were done in a private lab. Haematoxylin and Eosin (H & E) staining this process was achieved automatically by the H&E autostainer machine (leica Autostainer system CV5030). Immunohistochemistry (IHC): IHC is the localization of antigens or proteins in tissue sections by the use of labeled antibodies specific to the antigens and the antigen-antibody interactions are visualized by a marker such as fluorescent dye. IHC is used for diagnostic purposes and biological research as well as in drug development to test drug efficacy by detecting either the activity or the up or down regulation of disease targets. **14 Staining interpretation**

Positive control tissue: The positive control tissue is a known case of CRC that is positive for BRAF biomarker and was provided with the secondary detection kit. Positive reactivity was indicated by the presence of a brown reaction production at the site of the target antigen.

Negative control tissue: The negative control tissue was a known case of CRC that is negative for BRAF biomarker.

Statistical analysis: Statistical analysis and reporting of obtained data were carried out by using the Statistical Package for Social Sciences (SPSS V. 20). Note that all the collected adenoma samples showed low grade dysplasia after microscopic examination of tissue samples. Positive immunostaining with BRAF^{V600E} was observed in the cytoplasm of tumor cells. The color of stain for BRAF^{V600E} antibody was

brown.

Results:

In the carcinoma group, the patients age ranged from 40-90 years, with the highest percentage being in age range 70-79 years, (18cases, 30%), while in the adenoma group, the patient's age ranged from 30-90 years, with the highest percentage being among those 30-49 years (10 cases, 33.3%). Regarding the sex distribution, there were more males (40 cases, 66.7%) among the CRC cases compared to females (20 cases, 33.3%). There were 18 males (60%) among the adenoma cases compared to 12 females (40%). The tumor site for CRC cases, was the left colon in 39 cases (65%) compared to 21 cases (35%) in the right colon (ascending and transverse colon). As for the adenoma cases there were 22 cases (73.3%) in the left colon compared to 8 cases (26.6%) in the right colon (ascending and transverse colon). Most CRC cases were larger than 5 cm (36 cases, 60%), while 15 (50%) of the adenoma cases were 1-3 cm in size. Histologically, 50 of CRC cases (83.3%) were adenocarcinoma and 12 of the adenoma group were tubulovillous (40%). The pathological staging for CRC group revealed that 12 (20%) cases were stage I, 15 (25%) cases were stage II, 18 (30%) cases were stage III, and 15 (25%) cases were stage IV, while there histological grading revealed that 34 cases, (56.7%) were poorly differentiated. The BRAF^{V600E} pattern of expression revealed that 10 (16.6 %) CRC cases were diffusely positive, while none of the other 50 CRC cases and adenoma cases showed immunoreactivity to the BRAF^{V600E} biomarker as shown in table 1

Table 1: Distribution of selected clinicopathological parameters of colorectal adenoma and carcinoma cases

Parameter	Category	Carcinoma		Category	Adenoma	
		No.	%		No.	%
Age group (years)	40-49	6	10	30-49	10	33.3
	50-59	9	15	50-59	7	23.3
	60-69	15	25	60-69	6	20.0
	70-79	18	30	70-79	4	13.3
	80-90	12	20	80-90	3	10.0
Sex	Male	40	66.7	Male	18	60
	Female	20	33.3	Female	12	40
Site	Left colon	39	65	Lt colon	22	73.3
	Right colon	21	35	Rt colon	8	26.6
Size (cm)	< 5	24	40	<1	9	30
	> 5	36	60	1-3	15	50
				>3	6	20
Stage	I	12	20	Adenoma type		
	II	15	25	Tubular	8	26.6
	III	18	30	Villous	10	33.3
	IV	15	25	Tubulovillous	12	40.0
Grade / differentiation	Well	15	25			
	Moderate	11	18.3			
	Poor	34	56.7			
Histological type	Adenocarcinoma	50	83.3			
	Mucinous carcinoma	10	16.6			
BRAF expression	40-49	1	10			
	50-59	2	20			
	60-69	2	20			
	70-79	3	30			
	80-90	2	20			

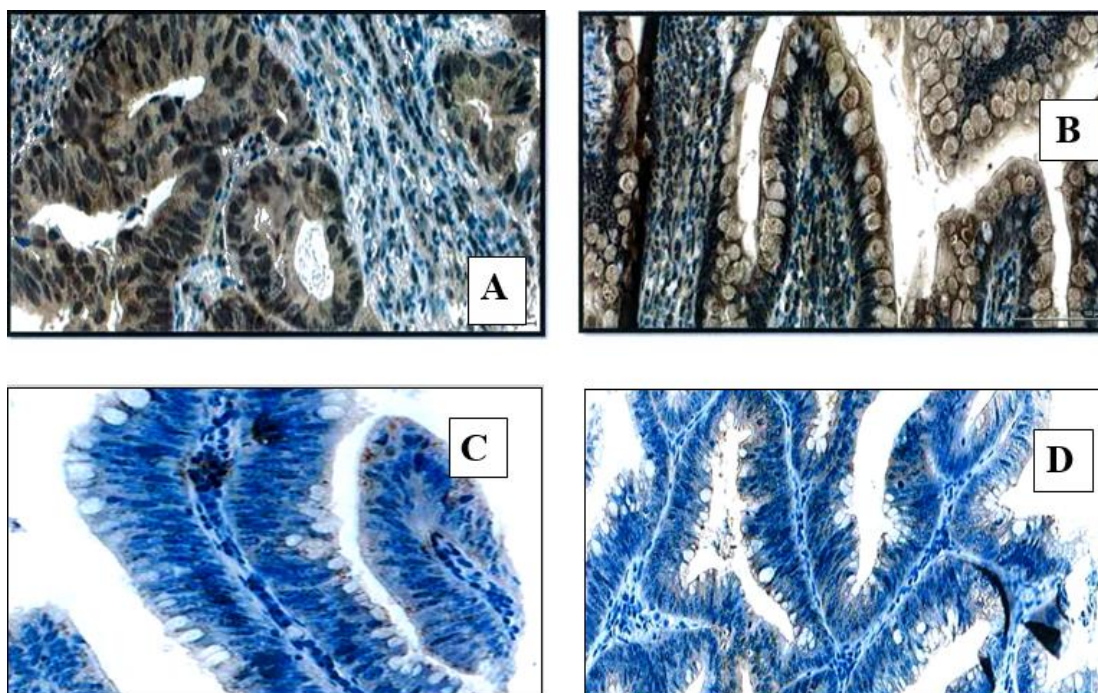


Figure 1: BRAF expression status

A and B: low power view 10x of well differentiated adenocarcinoma displaying positive cytoplasmic expression of BRAF^{V600E} (diffuse brown cytoplasmic staining pattern of tumor cells), **C:** Villous adenoma showing negative cytoplasmic expression of BRAF^{V600E}, **D:** Tubulovillous adenoma low power view 10x showing negative cytoplasmic staining pattern of cells (blue) low grade dysplasia

Table 2: The distribution of 60 CRC cases by demographic and tumor variables and by positive BRAF expression

Variable	Category	Total No.	% of total cases	Positive BRAF No.	% of Positive BRAF
Age	40-49	6	10	1	16.7
	50-59	9	15	2	22.2
	60-69	15	25	2	13.3
	70-79	18	30	3	16.7
	80-90	12	20	2	16.7
Stage	I	12	20	3	25.0
	II	15	25	1	6.7
	III	18	30	2	11.1
	IV	15	25	4	26.7
Sex	Male	40	66.7	5	12.5
	female	20	33.3	5	25.0
Site	Lt	39	65	6	15.4
	Rt	21	35	4	19.1
Size	5<	24	40	4	16.7
	5>	36	60	6	16.7
Grade/ differentiation	Well	15	25	4	26.7
	Moderate	11	18.3	0	0
	poor	34	56.7	6	17.6
Histological type	Adenocarcinoma	50	83.3	8	50.0
	Mucinous Ca	10	16.6	2	20.0
Total		60	100	10	16.7

Discussion:

The age distribution of the cases in the current study agrees with the American cancer society statistics (2014) which reported that (90%) of new cases and (93%) of deaths occur in people who are 50 years Old or older.15 Similar to other studies, our study revealed a higher frequency of CRC among males than females as reported by Abdulhussain and Othman (2012) who found that the CRC incidence

was (54.7%) among males and (45.3%) among females.16 Tumors located in the left colon were nearly double those located in the right colon in our study, which was also reported in previous studies from Iraq.17-20 In this study, adenocarcinoma comprised (83.3%) of cases, (56.7%) of them were poorly differentiated, grade III, which agrees with Gado et al, who found that

(91%) of their cases were adenocarcinoma.21 Histological grading and staging results in our study agreed with previous studies which stated that most cases were of moderate differentiation and comprising (66.7%) and (68.6%) respectively.16,22 Regarding BRAF expression, the BRAF biomarker was positive in 10 cases (16.6%) with diffuse cytoplasmic pattern of distribution. Any cytoplasmic staining was reported as positive result for BRAF, regardless of the intensity of staining, none of the other 50 CRC cases showed immunoreactivity to the anti-BRAF Antibody. This result agreed with another study published in 2017 and detected BRAF mutations in 137 of 1014 (13.5%) of patients with metastatic CRC.23

Regarding adenoma group, Sclafani et al. (2013) reported that nearly (6-85%) of hyperplastic polyps, (20-100%) of mixed polyps, and (20-100%) of serrated adenomas have been found to harbor a BRAF mutations compared to (0-5%) of conventional adenoma.24

Conclusions:

BRAF expression was higher in older patient's ages and higher tumor stages. BRAF^{V600E} mutation was positive in 10 (16.6%) cases of carcinoma group and associated significantly with different clinicopathologic variables while negative in adenoma group.

Authors' contributions:

Dr. Seror Abdul Kareem Turkan: Study conception, study design, data analysis, interpretation of data analysis, drafting the manuscript.

Dr. Thaeer Jawad Kadhim: Supervisor, support Dr. Seror Abdul Kareem Turkan to perform the analytic calculations and supervised the findings of this work.

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الكشف عن المؤشر الحيوي BRAF^{V600E} للمرضى بسرطان القولون والمستقيم باستعمال التقنية الكيميائية النسيجية المناعية / دراسة سريرية باثولوجية

الدكتورة سرور عبد الكريم تركان : بكالوريوس طب وجراحة عامة/ زميل المجلس العراقي للإختصاصات الطبية في علم الأمراض/ النسيج المرضي/ مركز اليرموك التدريبي
الأستاذ الدكتور ثامر الطائي : زميل المجلس العراقي للإختصاصات الطبية في علم الأمراض/ النسيج المرضي/ طبيب إستشاري في مركز اليرموك التدريبي

الخلاصة

المقدمة : إن سرطان القولون والمستقيم الرابع شيوياً بين سرطانات الجهاز الهضمي. لسيرين / ثريونين كابينيزات البروتين والتي تعمل RAF هو عضو من عائلة BRAF^{V600E} إن سرطانات القولون والمستقيم التي تحمل طفرة (ERK) / (MAPK). على تنظيم مسار مرتبطة بالأورام الإبتدائية في الجهة اليمنى وفي النساء الأكبر عمراً BRAF^{V600E} وتكون درجة نمو الورم نسيجياً متأخرة وتوقعات عواقب المرض سيئة.

الهدف من الدراسة: الكشف عن طفرة ال BRAF^{V600E} لعينة من المرضى العراقيين بسرطان القولون والمستقيم بواسطة التقنية الكيميائية النسيجية المناعية ودراسة علاقة المؤشر الحيوي BRAF^{V600E} مع المتغيرات السريرية الباثولوجية الأخرى مثلاً: عمر المريض ودرجة نمو الورم نسيجياً. **منهجية الدراسة:** في دراسة الحالات المتسلسلة هذه تم جمع ما مجموعه (9) من حالات القولون والمستقيم لعينة من المرضى العراقيين من المختبرات التعليمية لمستشفى اليرموك التعليمي في الفترة ما بين 20 سبتمبر 2019 الي 20 مايو 2020 والتي تشمل (60) حالة لسرطان القولون والمستقيم و (30) حالة من الورم العقدي الحميد. ثم تمت دراسة العينات في ما يخص تعبير المؤشر الحيوي BRAF^{V600E} وعلاقته ببعض المتغيرات السريرية الباثولوجية والتي تشمل عمر المريض, جنس المريض, موقع الورم, حجم الورم , النوع النسيجي للورم, درجة نمو الورم نسيجياً ودرجة إنتشار الورم في الجسم.

النتائج: فيما يخص حالات الورم الخبيث, التعبير المناعي للمؤشر الحيوي BRAF^{V600E} كان موجباً في 10 حالات (16.6 %) من حالات سرطان القولون والمستقيم وجميع حالات الورم الحميد أظهرت تعبيراً سالباً للمؤشر الحيوي BRAF^{V600E}.

الإستنتاج: التعبير المناعي للمؤشر الحيوي BRAF^{V600E} كان أعلى في المرضى الأكبر عمراً وفي الأورام الأكثر إنتشاراً في الجسم ولا يوجد تعبير مناعي للمؤشر الحيوي في حالات الورم العقدي الحميد.

الكلمات المفتاحية: CRC: سرطان القولون والمستقيم , BRAF^{V600E}: الجين الورمي الفايروسي المتماثل , MAPK: كابينيزات البروتين المنشطة بالميتوجين