# The Role of immunohistochemical Expression of monoclonal VE1 antibody in detecting the BRAF gene mutation in patients with colorectal carcinoma.

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## Abstract

**Purposes of study** : to evaluate clinical parameters and histopathological features of Colorectal carcinoma (CRC) and its association with BRAF mutation as immunohistochemical markers in Babylon province. **Methods**: This research is prospective study. The total patients are 42 Manual IHC staining procedure were done. Staining intensity were scored as: 0 ( negative ), 1 (weak diffuse staining in comparing to background staining ), 2 (moderate diffuse staining), and 3 (strong diffuse staining) . IHC scoring was regarding as positive when there was diffuse, homogenous and more than 80% of cytoplasmic staining area of cancer cells. Negative cases when there were absent staining or nuclear staining or weak isolated staining cells. Score 0 and 1 were consider negative and score 2 and 3 were positive. **Results**: Positive IHC staining of BRAF mutation was presenting more in older age group (65 ±15.23years) , male gender (60%) and left sided colon (60%) but there were insignificant association of these above parameters. Grade 2 and grade 3 of CRC was the highest frequency of positive BRAF cases (40%). Positive IHC staining for BRAF mutation expression is more frequency in T3, N1-2, and M0 stage, and stage III that shows 60%, 80%, 100%, 80% respectively. There were no significant association between BRAF IHC with TMN staging and grading systems. **Conclusion**: the current study found to be predominant in older age ( > 65 years old) , high grade (G2-3) and high stage (III). Parameters of high grade & stage associated with poor prognosis & high mortality outcome. BRAF gene IHC expression could be consider an independent bad prognostic factor for patients with CRC .

***Key words:*** *Colorectal carcinoma, BRAF gene, immunohistochemistry method.*

## Introduction

Colorectal carcinoma (CRC) is 3rd common malignancy in world(1) and one of leading cause of mortality in Western area of world(2) . Tumor-node-metastasis (TNM) staging system is remain as prognostic parameter for this cancer(3).

The distribution of this tumor is equal regarding sex and mean affecting age is between 6th and 7th decades of life(4).

Both chronic inflammatory bowel diseases and Schistosoma mansoni infection are causes of CRC (3) .

BRAF gene (v-Raf murine sarcoma viral oncogene homolog B) is an oncogene, undergo mutation, and found about 10%-15% of CRC(5,6). The common mutation in BRAF gene is V600E and account for about 80% (7). and found in colorectal adenocarcinoma (5%-15%), papillary type of thyroid cancer (45%), melanomas (40%-60%), serous type of ovarian cancer (35%), lung cancer (1%-3%) and other cancers(8).

CRC with BRAF oncogene mutation have recurrent association with poorly differentiation mucinous cancer and higher TNM staging system(9), and so, it can predict an essential role in treatment of CRC(10), and this BRAF mutation can distinguish between CRC of sporadic type

from hereditary non-polyposis type/Lynch syndrome[11].

Study aim was to evaluate clinical parameters and histopathological features of CRC and its association with BRAF as immunohistochemical markers in Babylon province.

## Method

### Patients samples

This research is prospective study. The patients with diagnosed CRC underwent clinical evaluation about age ,sex, metastasized CRC or not, if patients were taking chemotherapy or not, duration of CRC, and lastly, biopsy or total (or partial) colectomy.

These patients were selected from Al-Hilla Surgical Teaching Hospital, private Teeba Hospital, many private histopathological laboratory, and the specimens of CRC were partial or total colectomy, reported its large intestine site, and selected before chemotherapy. The paraffin embedded blocks of cancer were reviewed by two pathologists to ensure the diagnoses of CRC and were classified according to TNM staging system ($8^{th}$ edition, 2017)[12] and WHO grading system (2000)[13].

. The total patients (42) were collected between February/2017 to April/2019, 25 men and 17 women, and age ranged between 35-83 years old (median age 64).

### Immunohistochemistry (IHC)

The BRAF V600E kit protocol was Bio SB, Inc. , USA, BRAF VE1 antibody is Rabbit Monoclonal antibody (isotype IgG) that shows cellular cytoplasmic staining, Clone RM8, and Catalog No. BSB 2824.

IHC scoring of BRAF V600E was depend on percentage and intensity of staining, Staining intensity were scored as : 0 ( negative), 1 (weak diffuse staining in comparing to background staining ), 2 (moderate diffuse staining), and 3 (strong diffuse staining) . Positive control ( melanoma cancer has BRAF mutation) and negative control ( usually making by removing primary antibodies ) were used with each run of IHC procedure.

IHC scoring was regarding as positive when there was diffuse, homogenous and more than 80% of cytoplasmic staining area of cancer cells. Negative cases when there were absent staining or nuclear staining or weak isolated staining cells. Score 0 and 1 were consider negative and score 2 and 3 were positive [14,15]

## Statistical Analysis

SPSS software (version 22) was statistical program. Categories data were indicated as frequency and percentage and were assessment by using Pearson´s chi. Continuous data represented as range , median, mean ± SD, and were measured by Independent T Test if the data is normal distributed . *P* value < 0.05 was significant difference between two parameters.

## Results

A total 42 patients with CRC ( adenocarcinoma type ), 25 men and 17 women, mean age±SD ( 63.36±12.21), and were assessment by using IHC staining of BRAF mutation. General clinicopathological features of present study are illustrated in Table 1.

**TABLE 1: Clinicopathological basic characteristics in CRC patients (total n. =42).**

| Features | | | Total N (%) |
|---|---|---|---|
| Age (years old) | 35-54 | | 5(11.9%) |
| | 55-74 | | 27(64.3%) |
| | 75-83 | | 10(23.8%) |
| Gender | Male | | 25(59.5%) |
| | Female | | 17(40.5%) |

***Cont...*** **TABLE 1: Clinicopathological basic characteristics in CRC patients (total n. =42).**

| | | | |
|---|---|---|---|
| Tumor site | Left side colon | | 26(61.9%) |
| | Right side colon | | 10(23.8%) |
| | Rectum | | 6 (14.3)% |
| T stage | T1 | | 8(19%) |
| | T2 | | 18(42.9%) |
| | T3 | | 16(38.1%) |
| | T4 | | 0(0%) |
| N stage | N0 | | 6(14.3) |
| | N1-2 | | 36(85.7%) |
| M stage | M0 | | 39(92.9%) |
| | M1 | | 3(7.1%) |
| Tumor stage | I | | 3(7.1%) |
| | II | | 3(7.1%) |
| | III | | 33(78.6%) |
| | IV | | 3(7.1%) |
| Tumor grade | G1 | | 12(28.6%) |
| | G2 | | 20(47.6%) |
| | G3 | | 10(23.8%) |
| | G4 | | 0(0%) |

IHC staining procedure show 8 cases with BRAF staining and remaining 36 show no staining , 8 cases include strong intensity 2 cases, moderate intensity 3 cases, weak intensity 3 cases, and the staining percentage of these cases were between80%-95%. So, positive expression of BRAF IHC in CRC were represented always moderate to strong staining (5 cases).While, negative cases were including weak and no staining cases.

Positive IHC staining of BRAF mutation was presenting more in older age group (65 ±15.23years) , male gender (60%) and left sided colon (60%) but there were insignificant association of these above parameters as in Table 2.

**TABLE 2 : Association of clinical characteristics in CRC patients with IHC (total n. =42).**

| Clinical data | | | BRAF IHC N(%) | | P VALUE |
|---|---|---|---|---|---|
| | | | Negative | Positive | |
| Age mean±SD | | | 63±11.87 | 65±15.23 | #0.779 |
| Age (years old) | 35-54 | | 4(10.8%) | 1(20%) | ^0.834 |
| | 55-74 | | 24(64.9%) | 3(60%) | |
| | 75-83 | | 9 (24.3%) | 1(20%) | |
| Gender | Male | | 22(59.5%) | 3(60%) | ^0.982 |
| | Female | | 15(40.5%) | 2(40%) | |
| Tumor site | Left side colon | | 23(62.2%) | 3(60%) | ^0.487 |
| | Right side colon | | 8(21.6%) | 2(40%) | |
| | Rectum | | 6 (16.2%) | 0(0%) | |
| ^Pearson chi-Square | | | | | |
| #Independent Samples Test | | | | | |

Grade 2 and grade 3 of CRC was the highest frequency of positive BRAF cases (40%). Positive IHC staining for BRAF mutation expression is more frequency in T3, N1-2, and M0 stage, and stage III that shows 60%, 80%, 100%, 80% respectively.

There were no significant association between BRAF IHC with TMN staging and grading systems as in Table 3.

**TABLE 3 : Evaluation of histopathological features in CRC patients by IHC (total n. =42).**

| Features | | | BRAF IHC N(%) | | ^P VALUE |
|---|---|---|---|---|---|
| | | | Negative | Positive | |
| Tumor grade | G1 | | 11(29.7%) | 1(20%) | 0.657 |
| | G2 | | 18(48.6%) | 2(40%) | |
| | G3 | | 8(21.6%) | 2(40%) | |
| | G4 | | 0(0%) | 0(0%) | |
| Tumor grade | G1-G2 | | 29(78.4%) | 3(60%) | 0.365 |
| | G3-G4 | | 8(21.6%) | 2(40%) | |
| T stage | T1-2 | | 24(64.9%) | 2(40%) | 0.283 |
| | T3-4 | | 13(35.1%) | 3(60%) | |

| | | | | | |
|---|---|---|---|---|---|
| T stage | T1<br>T2<br>T3<br>T4 | | 8(21.6%)<br>16(43.2%)<br>13(35.1%)<br>0(0%) | 0(0%)<br>2(40%)<br>3(60%)<br>0(0%) | 0.405 |
| N stage | N0<br>N1-2 | | 5(13.5%)<br>32(86.5%) | 1(20%)<br>4(80%) | 0.697 |
| M stage | M0<br>M1 | | 34(91.9%)<br>3(8.1%) | 5(100%)<br>0(0%) | 0.509 |
| Tumor stage | I<br>II<br>III<br>IV | | 3(8.1%)<br>2(5.4%)<br>29(78.4%)<br>3(8.1%) | 0(0%)<br>1(20%)<br>4(80%)<br>0(0%) | 0.547 |

^Pearson chi-Square

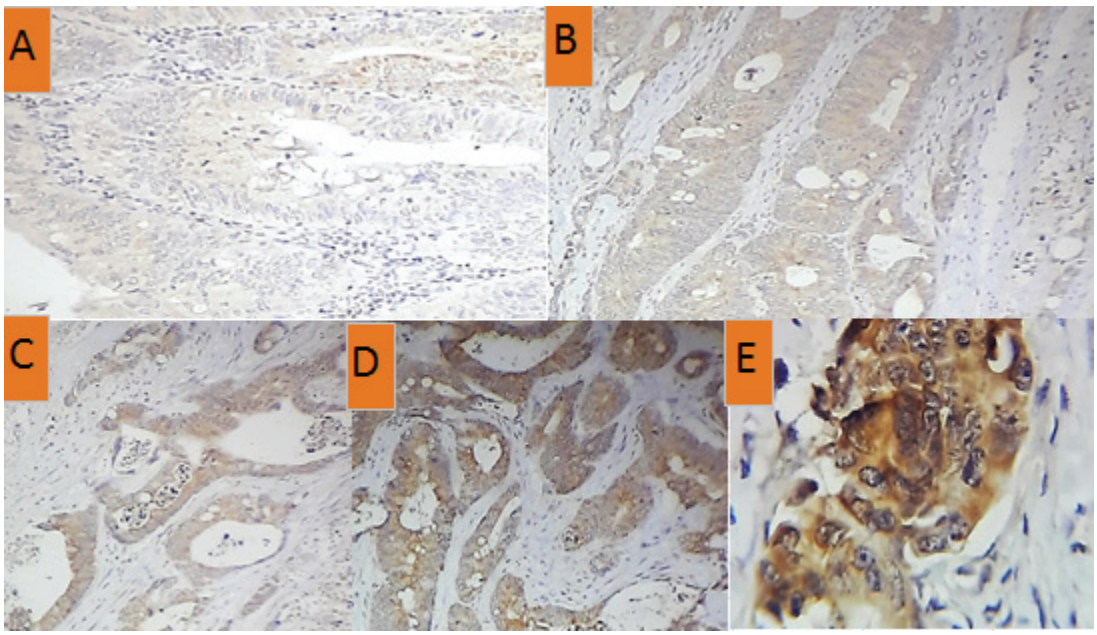


**FIG. 1 : BRAF IHC staining in the CRC. (A) It is showing negative staining at10X. (B) It is diffuse cytoplasmic and weak staining (10X), (C) and It is diffuse and moderate staining (X10) , (D) and (E) It has diffuse and strong staining at 10X and 40x respectively..**

## Discussion

The main objective of this study. Is to evaluate BRAF gene mutation prevalence by utilizing monoclonal VE1 AB in colorectal carcinoma & compare the outcomes with different clinical & histopathological features.

Many previous studies of BRAF gene showed good compatibility between IHC technique and genotype sequencing methods regarding many cancer types [16-23], but Adackapara et al[24] showed less compatibility (71% sensitivity and 74% specificity), this might be due to using manual IHC method with incubation overnight of IHC antibodies[25] .

BRAF mutation investigation is used as mandatory in practical laboratory[26]. Also, polymerase chain reaction technique required more time, need special structures, affected by method of preservation of formalin fixed paraffin tissue and tissue heterogeneity[20]. while, IHC is usually done in most pathological laboratories , less costly, and less complex procedure in compare to genotype procedure, lastly, genetic procedure for BRAF mutation can be regarded an critical method only when there are an equivocal IHC BRAF cases to confirm or exclude their positivity [27] .

Also, Sinicrope et al[15] used BRAF scoring as negative when there are nuclear staining or weak separated cancer cells with cytoplasmic staining, and positive scoring when there are 100% homogenous cytoplasmic staining area of cancer cells in 75% of patients or more than 70% of stained cells from total cases as scoring standard as in our research.

In the present study, there were 5 positive cases for VE1 (11.9%) most of them in older age and commonly in age group 55-74 years, male gender ,and left side of colon (tumor site), TNM stage (T3 , N1-2, M0), tumor grade (G2-3),however; no significant association between all these parameters and BRAF IHC results were observed.

Christian et al [28] (cohort 1) demonstrated that positive IHC BRAF cases (13.5%) were more in older age, female gender, left sided colon cancer, TNM stage (T3-T4, N0, and M0), and tumor grade (G1-2), All these factors (gender, tumor site, TNM tumor stage, and tumor grade) showed insignificant association with BRAF IHC.

Fiona et al [29] demonstrate that positive BRAF IHC (13.2%) expressed higher in female, right colon cancer, tumor grade 3, and tumor stage II-III.

Several studies of BRAF IHC showed result positivity more in older age, female sex, righted sided CRC, and more advanced tumor stage[30-33].

In conclusion, the current study is the first Iraqi study provide new information about the BRAF V600 gene mutation prevalence among Iraqi patients with CRC utilizing IHC technique, it found to be predominant in older age ( > 65 years old) , high grade (G2-3) and high stage (III). Parameters of high grade & stage associated with poor prognosis & high mortality outcome, then BRAF gene IHC expression could be consider an independent bad prognostic factor for those patients with CRC .

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