Original Article

Kras Immunehistochemical Expression In Colorectal Cancer

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Abstract

The goal of this study was to look at the expression of KRAS in colorectal cancer tissue samples and see how it correlated with clinicopathological characteristics. The study was designed in two ways: retrospectively and prospectively. Setting: Department of Pathology, Mosul University. From November 2021 to February 2022, 50 Formalin-fixed paraffin-embedded (FPPE) tissue blocks were collected from private pathology laboratories in Mosul, Iraq, from CRC patients who underwent colectomy.

Material and method : On paraffin-embedded tissue blocks, 50 instances of CRC were immunohistochemically stained with the KRAS polyclonal antibody. Data was gathered by retrieving clinical and pathology records. The immunohistochemistry analysis results were linked with the clinicopathological factors reported.

Results: During 8 monthes ,The study examined a total of 50 cases of colorectal cancer, and the various clinicopathological factors are described below .The mean age (SD) was (54.80) with range between 20-85 years, about (22)44% were female and28(56%) were male,the overall male:female ratio was 1.2:2 .According to microscopic types ,44(88%) cases of adenocarcinoma and 5 (10%) of mucinous and 1(2%) of neuroendocrine tumor. regarding to histological grading ,10(20%) cases were well differentiated G1 and 29(58%) for moderately differentiatedG2 and 11 (22%)cases were poor differentiated G3.Lymph node metastasis were positive in 23(46%) and free nodal tissie in 27(54%) of cases.

Conclusion: KRAS expression was found in advanced cancers in this investigation. The bulk of these cases were adenocarcinomas, with only a few showing mucinous histology. The current research suggests that established KRAS expression is typically detected in quickly dividing cells with the relationship of advanced malignancies

Keywords: colonrectal cancer ,kras, immunohistochemistry, EGFR polyclonal antibody

Introduction

Colorectal cancer is one of the diseases whose occurrence is expanding involving 11% of all malignant growth analyze. There were over 1.9 million new cases in2020. The majority of the increment is supposed to happen in nations with a high Human Development Index.

The International Agency for Research on Cancer (IARC) evaluated that the worldwide weight of colorectal disease will increment by 56% somewhere in the range of 2020 and 2040, to multiple million new cases each year. The

assessed expansion in the quantity of passings from the illness is considerably bigger, by 69%, to around 1.6 million passings worldwide in 2040

Concurring to GLOBOCAN 2020 information there is an expansive geographic variety in CRC rate and mortality among different nations of the world . It has been perceived that the main expansion in CRC rate and mortality happens in medium and high human improvement record (HDI) nations that are taking on the "western" method of life.

In Iraq, the incidence rate of Colorectal Cancer (CRC) in 2020 across all age groups is about 8.7 in both gender and 6.9 in female and 10.8 in male. In the GCC nations, CRC is viewed as the second most malignant growth among the two sexes with 2.3 increase and a 2.7 overlay expansion.

Long-term consumption of red meat or processed meat appears to be associated with an increased risk of colorectal cancer (CRC), particularly for left-sided tumors. High temperature cooking (eg, barbecuing, pan-frying) has been implicated as contributing to risk. (Akin & Tözün, 2014)).

Most of colon disease cases are inconsistent, this implies they are not connected with hereditary genetics or family history. Just 5% of yearly colon malignant growth are because of a "cancer gene" (Burt, 2000), in like manner, CRC is delegated as follows:. -1. Hereditary colon cancer 2. sporadic colon cancer.

Cancer development and progression is a multistep process based on somatic mutation accumulation and clonal selection in key cancer-related genes. Colorectal carcinomas are a well-known model for studying such alterations.

Colorectal carcinogenesis research has yielded important insights into the general pathways of cancer evolution. A route is defined by a group of genes that control a particular biological function. There are at least two different genetic pathways that have been described.

The first pathway is the traditional adenoma-carcinoma sequence, often known as the APC/-catenin pathway, which accounts for up to 80% of sporadic colon cancers. It is distinguished by chromosomal instability, which has resulted in the gradual accumulation of mutations in a variety of oncogenes and tumor suppressor genes that directly regulate cell birth and cell death.

The activity of the APC protein is closely related to that of another protein known as -catenin, which accumulates in the cytoplasm and stimulates cell growth . This causes a variety of physiological reactions, including the production of genes such as cyclin D1, which promote cell growth. This is followed by other mutations, including activating mutations in K-ras.

Kras mutation

The K-ras gene encodes a signal transduction molecule that oscillates between activated and inactive guanosine triphosphate (GTP)-bound states. K-ras mutations are stuck in an active state, which promotes growth and prevents apoptosis. The K-ras mutation usually occurs after the loss of APC. It is mutated in less than 10% of adenomas smaller than 1 cm, 50% of adenomas larger than 1 cm, and 50% of carcinomas. (Kumar and colleagues, 2010).

Kras mutation in colorectal cancer

Colon cancer progression can be classified into at least three phases. The initial stage is defined by the formation of a small, benign tubular adenoma or polyp with spontaneous K-ras mutation (s). The second stage is more aggressive and is frequently linked with patches of definite carcinoma cells that can develop into invasive malignancies in the third stage.

In colon cancer cases, K-ras gene status has become an important predictor of therapeutic outcome (Wójcik et al, 2009). Because colorectal cancer is a common neoplasia linked with EGFR1 (epidermal growth factor receptor gene1) pathway activation, novel and successful therapeutic options for this illness involve EGFR1 protein kinase inhibition. Colorectal cancer is often treated with two monoclonal antibodies targeting EGFR1. K-ras mutations are important in colorectal cancer patients' responses to therapeutic monoclonal antibodies. Khamba-Ford (2007); Amado et al. (2008); Lièvre et al. (2008); Ausch et al. (2009) In patients with K-ras wild-type mutations, the treatment impact of EGFR1 monoclonal antibody was greatly boosted compared to standard chemotherapy alone, whereas patients with a K-ras mutation had no effect.

As a result, detecting K-ras status with a K-ras strip assay kit prior to using EGFR monoclonal antibodies is now advised and deemed "best practice." Yuxia et al. (2010) At its annual meeting in 2009, the American Society of Clinical Oncology (ASCO) issued a Provisional Clinical Opinion outlining this recommendation, and the National Comprehensive Cancer Network (NCCN), another organization that develops clinical practice guidelines, has made this recommendation in their most recent guidelines for colon cancer care (Stintzing et al, 2009).

Why Is the KRAS Protein an Important Target to Study?

While wild-type KRAS normally promotes cell cycle progression, when activated to aberrant levels, it can also cause growth arrest, apoptosis, and replicative senescence. Cellular stress, ultraviolet or ionizing irradiation, thermal shock, and certain cytokines can all cause this. Oncogenic mutations in the KRAS gene impede GTP hydrolysis, resulting in RAS molecules that are persistently activated. Mutations in this gene have been demonstrated to increase metalloproteinase 2 (MMP2) expression in the matrix and increase cancer cell invasion.

Overexpression of this mutant version of KRAS also suppresses glycosylation of the integrin 1 chain, altering polarisation and increasing the adhesiveness of colon cancer cells. Furthermore, production of this oncogenic version of KRAS protein has been linked to increased carcinoembryonic antigen (CEA) expression and disruption of epithelial cell polarity (Jančík et al., 2010).

Importance of IHC(immunohistochemistry) in colorectal cancer:

KRAS mutations are seen in 14 percent to 50 percent of colorectal cancers (CRC) and are most commonly found in codons 12 and 13. For the treatment of CRC, anti-epidermal growth factor receptor (EGFR) antibodies such as cetuximab and panitumumab have been licensed. It binds to the extracellular domain of EGFR, inhibits ligand binding, and inhibits the downstream RAS-RAFMEK-ERK signaling cascade.

In patients with CRC, determining the KRAS mutation is now required before starting anti-EGFR therapy. KRAS and BRAF mutations are important in colorectal carcinogenesis and are linked to anti-EGFR resistance . Unfortunately, KRAS mutations have only been found at codons 12, 13, and 61 . As a result, increasing RAS testing in CRC to include additional mutations may improve prediction of effectiveness from anti-EGFR therapy.(Novrial, 2021)

However, due of anti-EGFR antibody resistance, this therapy is not indicated for patients with KRAS mutations (De Roock et al., 2010). As a result, KRAS status has emerged as an essential biomarker for patient selection.

PCR is a well-established test for detecting KRAS mutations since it has a high sensitivity even in samples with few tumor cells (Cree, 2016). In the clinical setting, PCR tests have some limitations, such as high cost and particular codon characteristics. Immunohistochemistry (IHC) has been proposed as a preliminary screening tool prior to genetic testing. It is a standard service in most pathology laboratories and is less expensive than molecular detection screening.

When it came to detecting MMR abnormalities, IHC outperformed molecular MSI testing in terms of sensitivity and specificity. Several prior investigations on the screening of ras gene aberrations, however, revealed inconsistent results.(Novrial, 2021).

Thus, In patients with KRAS positive protein expression, IHC as an alternative to molecular testing for rectal cancer may be effective as a prognostic and predictive marker in CRC.as increase testing volume will increase testing costs which may have economic implications.

In CRC, the prevalence of positive KRAS protein was similar to the prevalence of KRAS mutation in some study. However, one of the shortcomings of KRAS IHC tests was the use of polyclonal antibodies that were not selective to detect KRAS mutant. As a result, the creation of a monoclonal antibody directed against the altered KRAS domain is required. This could make it much easier to screen CRC patients for anti-EGFR therapy. IHC has the potential to become a useful tool in diagnostic and prognostic decisions in the future.

Aim of study:

- To identify the expression of Kras mutation in colorectal carcinoma cases by immunohistochemical study.
- . To compare the expression of kras mutation with some clinicopathological parameters.

Method

The Research Ethics Committee of the Faculty of Medicine at the University of Mosul gave its approval to this retrospective and prospective study. From november 2021 to February 2022, 50 Formalin-fixed paraffin-embedded (FPPE) tissue blocks from CRC patients who underwent colectomy were gathered from a private pathology laboratories in Mosul city \Iraq.The study consist of two parts molecular and immunohistochemistry to evaluate kras mutation in colorectal cancer samples.All participating patients provided demographic information (age at diagnosis, gender) and tumor topography (size, location nodal status, stage, and grade)and All blocks were inspected and the one with the best tumor (no necrosis, little mesenchymal tissue) was chosen for this study.

All paraffin blocks were staind with hematoxilin and eosin and reviewed for histopathological assessment.

IHC staining was conducted for all cases in PAR private hospital \Erbil city \Iraq. The immunostain kit from mybiosource by AL-shkairat establishment for medical supply.

Principle of procedure:

Immunohistochemical staining was performed on formalin fixed paraffine embedded (FPPE) tissue blocks.

4um sections of FPPE tissue blocks were transferred to positively charged slides. They were then deparaffinized, rehydrated,To reduce nonspecific background syaining due to endogenous peroxidase, blocked with hydrogen peroxide for 10 -15 minutes before antigen retrieval (Dako target retrieval solution, citrate buffer pH 6.0) at 100 0C. After a brief rinse in PBS about 2 time(2 minute fore each), the slides were incubated for (60-90 minute) at room temperature with the primary antibody against the KRAS oncoprotein (RDEFNab04641) KRAS polyclonal antibody: dilution 1:50).

After washing with PBS(2 minute), the slides were incubated for 30 minutes with labeled secondary antibody.The slides washed three times with PBS(2 minutes for each). The product was visualized (Dako) using diaminobenzidine (DAB)substrate as the chromogen. Incubated at room tempreture until suitable staining develops last about (5-8 minutes)

The slides were counterstained with hematoxylin and cleaned with distilled water and PBS once each. Finally, dehydrated slides with ethanol three times in different concentration as follow: washed slides in 75% ethanol Then 85% ethanol and finally 95% ethanol about 1 minute for each . after that slides washed in 2 changes of 100% ehanol rinsed about 1 minute each .cleared in xylene 3 times and also 1 minute for each , and mounted

under a coverslip.by usuing permanent mounting medium .allowed the slides to dry at room tempreture and analysed the result under light microscope .

Right colon, left colon, and rectum were the three groups into which the cancers were divided according to anatomical location based on the placement of the colorectum during embryonic development. Based on H&E-stained slides, the histological grading was assigned the letters G1, G2, and G3 for well, moderate, and poor.(ava ismael tahir, 2012)

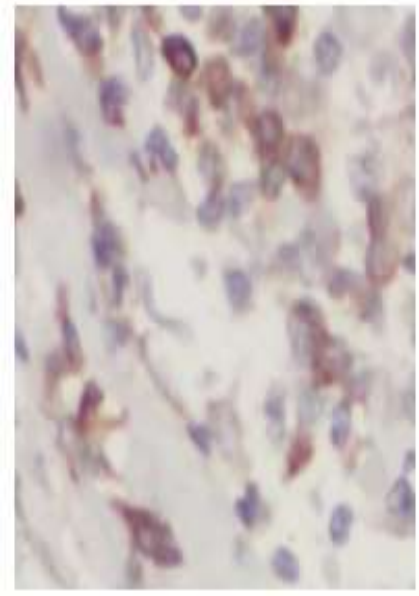
Immunoreactivity was determined by calculating the percentage of positive tumor cells tumors were classified as KRAS negative if less than 10% of cancer cells were unstained, and KRAS positive if more than 10% of cells were immunostained.

A pathologist scored the cytoplasmic IHC staining of K-ras protein subjectively under a light microscope, and the percentage of stained tumor cells (brown color) was expressed using the previously established criteria of Akkiprik et al.(Elsabah & Adel, 2013) as follows:

3(+) if the majority of cells (>75%) were stained considered as strong

2(+) if 25-50% of cells were stained moderate and 1(+) if the staining was focal (25%) considered as weak and non-stained cells were considered negative, and if <10% of cells were stained. (Pandey et al., 2020)

Immunohistochemistry of paraffin-embedded human lung cancer tissue slide usingRDEFNabO4641 (KRAS Antibody) at dilution of 1:100 used as positive external control. negative controls were cases where the primary antibody was omitted.



Immunohistochemistry of paraffin-embedded human lung cancer tissue slide using RDEFNab04641(KRAS Antibody) at dilution of 1:100

All statistical analyses were performed using the IBM-Statistical Package for Social Sciences (SPSS) analysis softwere ,version 25 . The Chi-square test was used to compare the category variables. All P values were computed using two-sided testing, and P 0.05 was regarded as significant. Comparing the clinicopathological parameter of cases with the expressed KRAS protein .

The Chi-square test was used to determine whether there was a significant relationship between the various clinicopathological factors and K-ras immunostaining data.

The results:

During 8 monthes ,The study examined a total of 50 cases of colorectal cancer, and the various clinicopathological factors are described below .The mean age (SD) was (54.80) with range between 20-85 years, about (22)44% were female and28(56%) were male,the overall male:female ratio was 1.2:2 .

According to microscopic types ,44(88%) cases of adenocarcinoma and 5 (10%) of mucinous and 1(2%) of neuroendocrine tumor. regarding to histological grading ,10(20%) cases were well differentiated G1 and 29(58%) for moderately differentiatedG2 and 11 (22%)cases were poor differentiated G3.

Lymph node metastasis were positive in 23(46%) and free nodal tissie in 27(54%) of cases.

IHC results:

Among the 50 patients examined with kras immunostaining, 28 (56%) were positive . the positivity eas either strong +3 in 12 (24%) cases or moderate in +2 in 16(32%) cases .the other cases were 22(44%) with score 1 or unstained.

Clinical relationship :

Our research found no statistically significant relationship between age, gender, and kras immunostaining. Positive results were found in 28 (56% of the cases), with 22 (44%) being adenocarcinoma cases (11 strong and 11 moderate)with significant correlation between the kras immunostainig and adenocarcinoma tupe.(p value 0.001) . while the other 22 cases were negative There were 5 mucinous tumors, 1 (2%), was positive, and 1 neuroendocrine tumor was negative. Because the number of cases of mucinous and neuroendocrine tumors was small, no statistical difference was determined.(table 1).

Only four of the ten (20%) well differentiated G1 were positive (4 strong and 0 mild), while the others were negative. while , 15(30%) from 29(58%) of moderately differentiated G2 were positive (8 strong ,7 moderate staining). Six of eleven cases of poorly differentiated adenocarcinoma G3 were found to be positive (2 strong,4 moderate).while the remaining cases were naegative .there was no significant relations between the grade and the kras immune stain .with p value :0.475.

Concerning nodal metastasis, immunostain was reactive in 12 (24%) of the 23 (46%) cases of positive metastasis (4 moderate,8 strong).

while the remaining 11 cases (22%) were negative (7 unstained and 4 weak staining 1).

In another case of free nodal metastases 27 (54%), were 11(22%) positive reactions (7 moderate,4 strong), and 16 cases(32%) were negative reactions (12 unstained, 4 weak stainings). without significant relation between nodal status and Kras overexpression with p value 0.080.

Regarding tumor location, around 21 (42%) cases were in the left colon, with 12 Kras positive reactions (6 moderate stainings, 6 strong), and the remaining 9 cases were negative reactions (8 unstained,1 weak staining).

10 (20%)in the right colon,4 positive Kras reactions (1 moderate,3 strong stainings), the remaining 6 cases were negative with (6 unstained,o weak).

the other 19 (39%) cases were located in the rectum with positive Kras reaction in 8 (16%) (4 moderate,4 strong). the remaining 11 cases of 19 had a negative reaction (5 unstained,6 weak stainings). there was no significant relationship between the site of the tumor and Kras status p value 0.194.

In terms of tumor size, the total cases were classified into two groups: >5 cm and <5cm, with <5cm being the most common, 36 (72%) cases, out of 16 (32%), showing a positive Kras reaction (6 moderate,10 strong). The remaining 20 (40%) instances had negative Kras responses (14 unstained,6 weak stainings).

The other group was >5cm. There were 14 (28%) cases, 9 of which had a positive reaction (5 moderate, 4 high), whereas the remaining 5 had a negative reaction (4 un staind and 1 weak stain). There was no relation between the size and the kras immunhistochimistry with p value 0.792.

In terms of stage, Kras staining was positive in approximately 23 (46%) cases, which were distributed as follows: approximately 22 (44%) cases were stage 2 and 10 (20%) cases of positive Kras expression (7 moderate, 3 strong) whereas the other 12 (24%) cases of negative Kras staining (10 unstained,.2 weak staining 1+).

Concerning stage 3, approximately 20 (40%) cases had an equal distribution of positive and negative staining but differed in degree of the score as follows: Ten (20%) cases had positive staining (7 moderate, 3 strong), while the remaining ten cases had negative staining (5, moderate,5 strong).

the least number of case distributions were stages 1 and 4, stage 1 had 5 (10%) cases with 2 positive Kras stainings (1 moderate,1 strong). and 3 cases of 0 scores (unstained). On another hand stage, 4 had 1 positive expression of strong staining and 2 un-stained cases were considered negative. With no significant relation between stages and kras staining ,p value 0.541.

Table 1 :relations between kras immunostaining and clinicopathological parameter .

			negative		positive		
variables	Frequency NO.	%	0	1	2	3	P value
Age(yrs)							
20-30	5	10	2(4%)	0(0%)	3(6%)	0(0%)	0.465
31-40	4	8	0(0%)	2(4%)	0(0%)	1(2%)	
41-50	7	14	1(2%)	1(2%)	2(4%)	3(6%)	
51-60	14	28	5(10%)	1(2%)	5(10%)	3(6%)	
61-70	10	20	5(10%)	2(4%)	2(4%)	1(2%)	
>70	11	22	6(12%)	1(2%)	0(0%)	4(8%)	
Gender							
male	28	56	9(18%)	5(10%)	4(8%)	10(20%)	0.703
female	22	44	8(16%)	2(4%)	7(14%)	5(10%)	
Size(cm)							
>5	14	28	4(8%)	1(2%)	5(10%)	4(8%)	0.792
<5	36	72	14(28%)	6(12%)	6(12%)	10(20%)	
Site							
Rt.colon	10	20	6(12%)	0(0%)	1(2%)	3(9%)	0.194
Lt.colon	21	42	8(16%)	1(2%)	6(12%)	6(12%)	

rectum	19	38	5(10%)	6(12%)	4(8%)	4(8%)	
Grade							
well	10	20	4(8%)	2(4%)	0(0%)	4(8%)	0.475
moderate	29	58	9(18%)	5(10%)	7(14%)	8(16%)	
poor	11	22	5(10%)	0(0%)	4(8%)	2(4%)	
Stage							
1	5	10	3(6%)	0(0%)	1(1%)	1(2%)	0.541
2a	10	20	4(8%)	1(2%)	3(6%)	2(4%)	
2b	8	16	3(6%)	1(2%)	3(6%)	1(2%)	
2c	4	8	3(6%)	0(0%)	1(2%)	0(0%)	
3b	16	32	5(10%)	3(6%)	2(4%)	6(12%)	
3c	4	8	0(0%)	2(4%)	1(2%)	1(2%)	
4	3	6	2(4%)	0(0%)	0(0%)	1(2%)	
LN.							
positive	23	46	7(14%)	4(8%)	4(8%)	8(16%)	0.080
negative	27	54	12(24%)	4(8%)	7(14%)	4(8%)	
Histologicaltype							
adenocarcinoma	44	88	15(30%)	7(14%)	11(22%)	11(22%)	0.001
mucinous	5	10	3(6%)	1(2%)	0(0%)	1(2%)	
Neuroendocrine tumor	1	2	1(2%)	0(0%)	0(0%)	0(0%)	

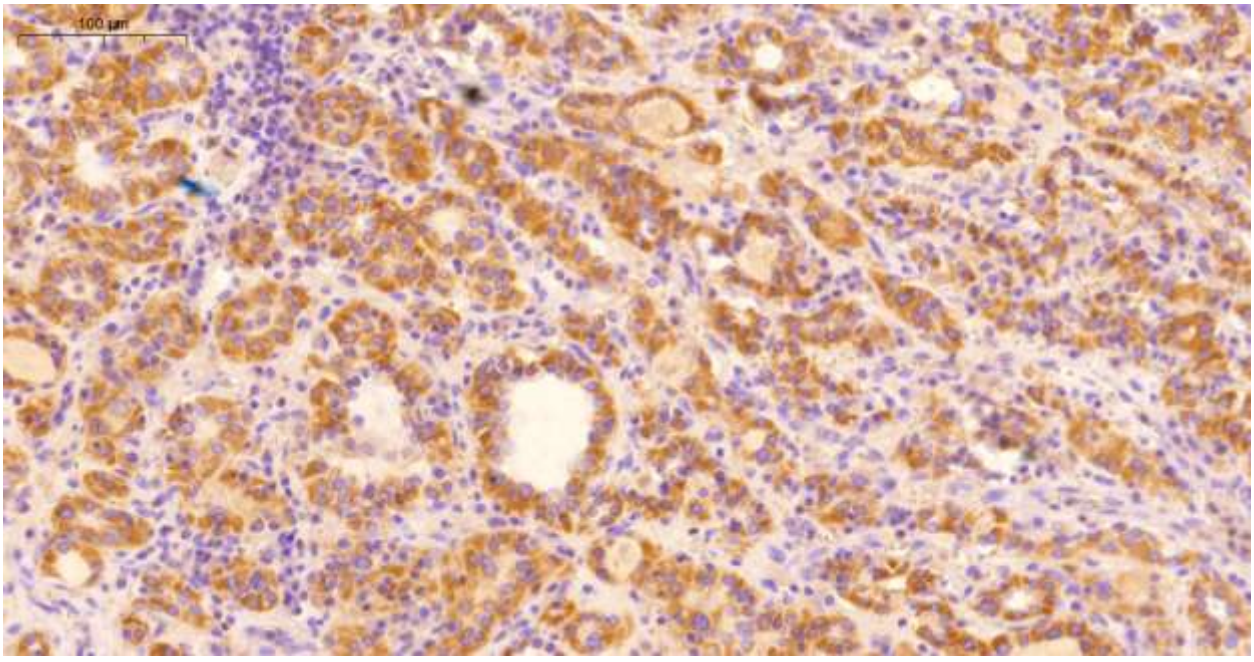
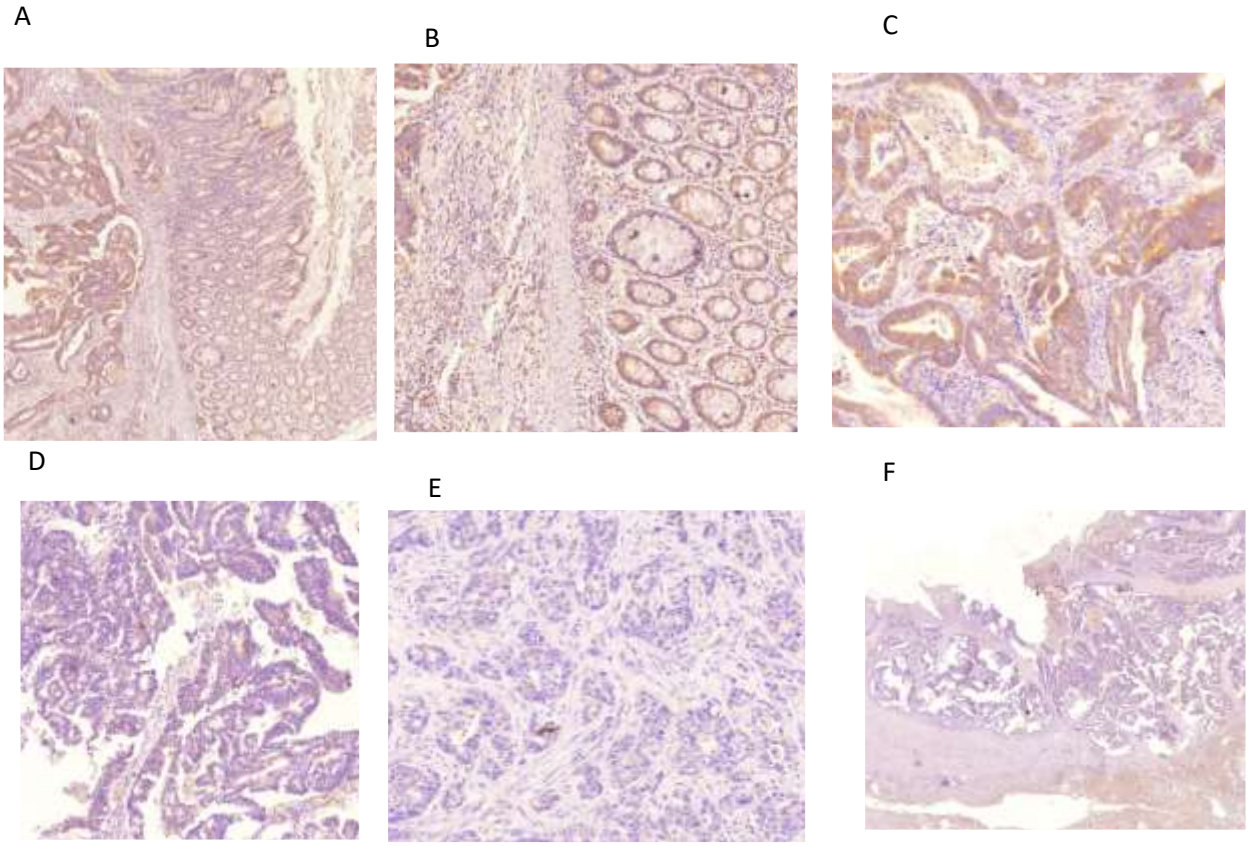


fig. 1 :adenocarcinoma with positive cytoplasmic staining(X 40)



A :represent positive kras expression in tumor tissue (10x)

B: positive kras exoression in tumor tissue (40x).

C : positive kras expression with strong intensity in moderately differentiated colorectal cancer tissue (40x).

D:negative expression (weak staining intensity) in moderately differentiated colorectal cancer tissue (40x)

E:poorly diffrentiated colorectal cancer with negative kras expression

F:moderately differentiated tumor with positive kras expression with moderate intensity(40x).

Discussion

Colorectal cancer develops in a multistep process caused by the accumulation of various genetic abnormalities. The most significant are RAS and RAF, which have been identified as critical intermediates in the RAS-mediated signaling cascade**.**(Kanik et al., 2018)**.**

The purpose of this study was to quantify the prevalence of colorectal malignancies seen mosul and to assess the overexpression of the KRAS proto-oncogene as an established marker in tumors.

The determination of the KRAS mutation is required for treatment with anti-EGFR therapy in patients suffering from CRC.

Increased testing volume, on the other hand, will increase Costs of testing that may have an economic impact, As a result, the morphological investigation was followed by protein identification using IHC in the hopes of providing an alternative to molecular screening. IHC of KRAS may be beneficial as a prognostic and predictive marker in CRC, as KRAS positive protein expression has been linked to CRC disease aggressiveness.(Novrial, 2021).

In this study, we discovered KRAS protein expression in28 case o(56%)of CRC patients. The remaining 22 cases (44%) had negative expressions (unstained or weak). This result was close to study in 50 CRC patients by Zlatian et al(Embryol, 2015). discovered KRAS immunopositivity in 52% of cases and found that KRAS expression was significantly enhanced in poorly differentiated adenocarcinoma (p=0.037) The percentage was extremely close to that reported in our study, probably due to faster replication and a greater possibility to accumulate oncogenic mutations. . another research, also close to our research, the Egyptian study's 42.3% (Elsabah & Adel, 2013), but lower than the Chinese study's (71%). and also lower than the 80% kras positive found in a Pakistani study (Wasti et al., 2022**).**

Several investigations found upregulation of KRAS, BRAF, MEK, and ERK using IHC and q PCR, implying that IHC is an equally effective diagnostic method.(Wasti et al., 2022).

These variations in mutations positivity ranging from 31% to 80%. ((Wasti et al., 2022),(Novrial, 2021)). So These changes were identified as a result of many flaws in mutation analyses, including codon specific mutation locations, ethnic variations, food, and lifestyle factors.

Our study found that 44% of Kras positive expressions were adenocarcinoma as histological variants , which is similar to (Elsabah & Adel, 2013) 50%. . This could be owing to the high prevalence of Kras mutation in Arabic countries(AlZaabi, n.d.) The prevalence of KRAS in the Arab population has been found to range between 30% to 50%, which is comparable to data from the Western population. , or it could be related to the sampling collection, which found that 88% of cases were adenocarcinoma.

In the current study, we found a link between KRAS overexpression and a variety of clinicopathological factors such as age, gender, tumor localization, tumor grade, and histological variations. The patient's age, gender, and tumor site did not show any significant link with KRAS overexpression and these parameter

Our findings are consistent with recent studies that found no significant connection between age, gender, and tumor site , size and KRAS overexpression in colorectal cancer patients. So this finding agree with study (Xie et al., 2019),that consider no significant relation between site of tumor and kras expression status .

In terms of grade, there was a descriptive relationship in moderate grade and kras positive expression as a result of the majority of moderate grade among cases (58%). and had a 30% kras positive expression. this is consistent with the research of(Elsabah & Adel, 2013).

The most important factor influencing prognosis is tumor stage, which is determined by the depth of tumor invasion, lymph node metastasis, and distant metastasis. KRAS, protein expression in CRC tissues has not been observed for significant relation but descriptive mainly in stage 2 and 3 in this research, so this agree with study of (Wan et al., 2019).

KRAS mutant tumors are thought to be linked to more advanced stage CRC. Studies conducted in the Arab countries on patients at various stages of CRC found no such link. As a result, the predictive significance of KRAS mutation varied amongst research. In a Saudi Arabian study, it was linked to a poor prognosis and outcome regardless of tumor stage.(Novi SB, 2018).

Immunohistochemistry is a powerful method that provides morphological support for phenotypic characterization, particularly the discovery of heterogeneity within tumor cells. Its validity, however, is dependent on the specificity of antibodies, which must detect just the altered protein..

In terms of laboratory time, immunohistochemistry can be completed in one working day, whereas molecular procedures require at least two working days.

Immunohistochemical evaluation may also be useful in circumstances where the quality of the material is insufficient for molecular analysis: the presence of dispersed cells in the specimen can be used to diagnose malignant tumors. Molecular approaches, on the other hand, require at least 5% of tumoral cells in the analyzed sample.

despite variable results among new research on Kras in CRC, and this could be explained by variation in study design, particularly study parameters related to sample size, ethnic group, modality of testing the KRAS mutation, and timing of the reported tumor stage, whether the reported stage was at the time of diagnosis or data collection.and in other hand using poly clonal antibody wich can react with both wild and mutant kras protein and consider as limitation in our study, The prevalence of positive KRAS protein in CRC was

relatively the same as the prevalence of KRASmutation as in (Piton et al., 2015).

Conclusion :

KRAS expression was found in advanced cancers in this investigation. The bulk of these cases were adenocarcinomas, with only a few showing mucinous histology. The current research suggests that established KRAS expression is typically detected in quickly dividing cells with the relationship of advanced malignancies.

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