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Molecular assessments of BRAF, KRAS and relationship with a clinic opathological feature in Sporadic Colorectal Cancer

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ABSTRACT
Background: One of the most prevalent kinds of cancerous tumours seen all over the globe is the sporadic type of CRC, which is also referred to as CRC. Alterations in the oncogenes have a key role in the regulation of cell division, particularly in the context of tumours. These adjustments trigger cell division by sending signals from the exterior of the cell to the nucleus, which is located in the centre of the cell.
Objective: The aim of this investigationis to assessment related between the mutations in Kirsten rat sarcoma virus(KRAS), serine/threonine-protein kinase B-Raf (BRAF) genesand sporadic CRC(CRC) disease.
Methods: A total of 47 samples from patients (males 53.2% - females 46.8%)with CRC and conventional polymerase chain reaction (cPCR)were used to test all specimens. The study groups were classified according to age into two groups, 1st (G1), patients below 50 years old and 2nd (G2),including ages above 50.The mutation in the BRAFand KRASgenes was selected at 12,13, and 15 codons. Tumours were classified into well, moderate and poorly differentiated, and the site of the tumour was into the right, left,and rectum. Data analysis was performed using the SPSS and GraphPad prism with a significant rate at P. value <0.05.
Results: The male and female patients of this study were significantly varied between groups of gender 26 male-21 female (P=0.0149) and age 18 G1 <50 -19 G2>50 years old (P=0.0488). Distribution of the grades in this study was significantly different between groups, well, moderate and poorly differentiated (7, 25 and 15, P.value= 0.0107), and similar findings in the statistics of the tumour site right, left, and rectum (24, 17 and 6, P.value=0.0252). This study recorded 61.7% mutation in KRAS, while 25.53% mutation in BRAF correlated with CRC.
Conclusion: we concluded that mutations in the KARAS and BRAF genes mightcorrelate with the CRC incidence in patients from Iraq.

Keywords: Colorectal Cancer, mutations, colon, rectum, exons, KARAS, BRAF

INTRODUCTION

Sporadic CRC is one of the kinds of the illness that isdiagnosed with the greatest frequency in the nations of the world with 70% from other CRC. There were almost 1.2 million persons in every region of the world who were given a diagnosis of CRC, and the death rate associated to the disease was roughly 36 percent (1,2).The disease affects somewhat more men than it does women, and the median age at which sporadic colon cancer is detected in patients is 70 years old; as a result, it is often considered to be a health problem that is more prevalent among older people (3).More than ninety percent of instances of CRC are identified as sporadic, which suggests that the individuals who are affected do not have a prior diagnosis of colon cancer in their family (4).
The KRAS gene is an oncogene that, when translated into a protein, results in the creation of the KRAS protein, which is a membrane-bound G protein of moderate size. The KRAS protein is responsible for the progression of many types of cancer. This protein is to blame for the development of a wide variety of various cancers (5). KRAS protein, which was activated in a process known as receptor tyrosine kinase activation, plays an essential part in the control of cell division by transferring signals of external proliferation to the nucleus. This mechanism is responsible for the development of cancer and other diseases. The development of cancer may be traced back to this particular mechanism (6). An operation very much like this one take place in the process of cell division. Increased tyrosine kinase activity is produced by the KRAS gene when mutations take place in the gene, notably in codons 12 and 13. These codons are particularly susceptible to mutation. This in turn leads to the encouragement of cell transformation, which in turn leads to the development of aggressive tumours and resistance to chemotherapeutic and anti-EGFR-targeted pharmacological therapies (7). It has been shown that mutations in the KRAS gene, which are what allow the gene to become active, are present in around 35–45

percent of CRC samples. These mutations have been linked to the development of the disease. These alterations are related with inadequate responses to treatment (8).In spite of the fact that the presence of a KRAS gene in its wild type is not a good indicator of a favourable response to EGFR-targeted therapy, recent research has shown that a BRAF genotype in its wild type is also required for anti-EGFR-based treatments to be effective. This is due to the fact that KRAS is a gene that is involved in the development of cancer (9,10).

BRAF is a gene that encodes a protein kinase that is involved in the mitogen-activated protein kinase (MAPK) signalling cascade. It is a member of the RAS/RAF family of genes. Additionally, it is recognised by the name v-Raf murine sarcoma viral oncogene homolog B1. The initial finding made by BRAF came from the study of sarcomas seen in mice (11,12)BRAFoperates as a direct effector of RAS and helps to the creation and survival of tumours. This contribution is made possible by the activation of MEK (MAPK/ERK kinase). BRAF acts as a direct effector of RAS through this pathway, which explains how it works (13,14). Most frequent activating mutations of BRAF found in CRC almost invariably result in valine substituting glutamate at residue 600 (BRAF V600E) and occur at a frequency of 10–15% (15).

CRC is the end result of a multi-step carcinogenic process that involves the accumulation of epigenetic and genetic changes (16).These alterations, which are required for the development of squamous cell carcinoma, include mutations in the KRAS and BRAF genes (17).The BRAF proto-oncogene is both recruited and active due to the GTPase that is encoded by the KRAS proto-oncogene, which in turn is caused by the KRAS proto-oncogene. This is because of a gene called KRAS, which is a proto-oncogene. This specific route is affected by the epidermal growth factor receptor/mitogen-activated protein kinases pathway, which is also referred to as the MAPK pathway (18).However, prior to the administration of anti-EGFR targeted drugs for patients suffering from CRC, mutation analysis of the KRAS and BRAF genes, which are both a component of the EGFR/MAPK pathway, has become an essential step (19).

There is a relationship between the development of cutaneous squamous cell carcinoma and the gradual variations in genetics and epigenetics, which have an effect on apoptosis, proliferation, and hemostasis. This relationship is supported by evidence CRC. The following are some more potential courses of action to take: Additionally, mutations within the proto-oncogenes of KRAS and BRAF, both of which are positioned downstream of EGFR along this pathway, might be responsible for the constitutive activation of this receptor. Both of these proto-oncogenes are present along this route (20). These two proto-oncogenes may be found in close proximity to one another along this route. If this were the case, it would indicate that the receptor is always active; however, this is not the case. It is estimated that between 35 and 40 percent of colon tumours include a KRAS mutation. Approximately two-thirds of these mutations are found in codon 12, while the remaining one-third is located in codon 13. In patients who have advanced colon cancer, the presence of a KRAS mutation is indicative of resistance to the treatment with anti-EFGR monoclonal antibodies. (mAbs) (20, 21).

In this study, we studied the potential of a link between KRASand BRAF genotypes and epigenetic factors. In addition, we differentiated clinicopathological and morphological parameters, such as the gender and age of patients, as well as histology data. The aim of study is to investigate Molecular assessments of BRAF, KRAS and relationship with a clinicopathological feature in CRC.

MATERIAL AND METHODS

Sampling

were obtained from a number of hospitals around the country, both public and private, in addition to the general hospital. Specimens obtained after surgery for the excision of tumours were preserved with 10% buffered formalinized saline, and paraffin embedded tissue blocks were prepared for histological and molecular diagnostic procedures using DNA extracted from FFEP. These procedures were carried out with the help of the specimens. These operations were carried out using tissue blocks that had been encased in paraffin.

Histology preparation

The slides that were cut from the paraffin-embedded and fixed tissue blocks were stained with the haematoxylin and eosin stain so that they could be examined more closely. After that, the slides were looked at by a pathologist who had a great deal of experience.

DNA extraction

The DNA FFPE tissue kit has fifty distinct reactions. Mineute columns, kits. An examination of genetic material via nanodrop technology In order to obtain accurate results from PCR-SSP using DNA with an A260/A280 quotient of 1.6 or above, the DNA must have a value of 1.5 for the A260/A280 ratio, which represents the proportion of protein present in the DNA preparation. This is necessary in order to ensure that the PCR-SSP procedure is carried out correctly. It is anticipated that more than fifty percent of the cells present in the sections of tumour tissue would exhibit neoplastic characteristics (22).in order to avoid generating incorrect negative results. Internal control, DNA extracted from FFPE may have varying degrees of degradation and may include PCR inhibitors; as a result, we advise doing an initial quality control test to determine whether or not a sample is

correct. DNA may be used to determine the optimal quantity required for the amplification process. Amplification of the b-globin gene is required in order to achieve this objective. The b-globin gene is a good choice for use as a target for the control PCR since it is constantly present (it does not go through deletions), and it does not fluctuate from cell to cell (23).DNA extracted from healthy human lymphocytes at a concentration of 50 ng/ml used as the standard for the b-globin assay (24).Thermal cycling: 94 degrees Celsius 10' plus five times (94 degrees Celsius 60", 55 degrees Celsius 60", and 72 degrees Celsius 60") plus thirty-five times (94 degrees Celsius 30", 55 degrees Celsius 30", and 72 degrees Celsius 30") + five times (72 degrees Celsius 5'). in each gene while maintaining the same concentration of b-globin PCR reaction mix.
In order to analyseKRAS, the PCR reaction is performed with a total volume of 25 l. 20 microliters of Mister mix, 1 microliter each of forward and reverse primer (Table 1)at a concentration of 30 pmol/ml, 1 microliter of diluted sample DNA at a concentration of 50 ng/ml, and the remaining volume should be filled with water. Thermal cycling: 95 degrees Celsius for four minutes, followed by (54 degrees Celsius for thirty seconds, 72 degrees Celsius for sixty seconds, and then 35 degrees Celsius for seven minutes at 72 degrees Celsius. During the time that we spent in BRAF Thermal cycling: 95 degrees Celsius for four minutes, followed by96 degrees Celsius for 30 seconds, 52 degrees Celsius for 30 seconds, and 72 degrees Celsius for 60 seconds), 35 degrees Celsius, and 72 degrees Celsius for seven minutes. After being electrophoresed in agarose gels at a concentration of two percent and being stained with ethidium bromide, the results of a polymerase chain reaction were seen under ultraviolet light. The gels had been electrophoresed in agarose.

Table 1. Primers sequences genes in study.

Site	Gene orLocus	Primer Sequence (5–3)	Size, bp
EXON 12,13	KRAS-F KRAS-R	5'-AAGGCCTGCTGAAAATGACTG-3' 5' CAAAGAATGGTCCTGCACCAG-3'	173bp
EXON 15	BRAF-F BRAF-R	5'CTCTTCATAATGCTTGCTCTGATAGG3' 5' TAGTAACTCAGCAGCATCTCAGG-3'	250bp
	b-globin F b-globin R	5'ACACAACTGTGTTCACTAGC3' 5'GAAAATAGACCAATAGGCAG3'	167bp

Statistical methods
The chi-square test was used in order to assess the significance of the reported variations in proportions. When there was a cell having a value that was less than 5, the exact Fisher test was employed. SPSS version 15, which was also used for the execution of the chi-square and Fisher exact tests, was the programme that was employed to input data into the computer. P values that were deemed to be statistically significant were those that were either equal to or lower than the value of 0.05(25).

RESULTS
This research included a total of 47 patients who were diagnosed with CRC. Of these 47 patients, 26 (or 55%) were men and 21 (or 45%) were females. The patients' ages ranged from 37 to 72 years, with a mean of 54.5 years, and the ratio of females to males was 1:1.2. The ages were divided into two groups: the first group included people who were below the age of 50 (38.29%), and the second group had people who were beyond the age of 50 (61.70%). Table 2 provides a summary of these two groups.It was noted that 14.89 percent of cases of CRC were well differentiated, 53.19% of cases were moderately differentiated, and 31.91% of cases were poorly differentiated. These percentages refer to the grades of colorectal carcinoma. Patients who had CRC were separated into three categories based on the location of the tumour: patients with CRC in the rectum made up 12.76% of all cases, patients with CRC in the right colon made up 51.06% of all cases, and patients with CRC in the left colon made up 36.17%.With considerable differences between gender, age, morphological characteristic, and place of infection., age, clinicopathologic and site of tumorCRC (Table 2, Figure 1).

Table 2. Distributions of patient's gender, age, clinicopathologic and site of tumor CRC

Gender	Status	No. (%)	P value
	Female	21 (44.68%)	0.0488
	Male	26 (55.32%)	
		47	
Age	Group 1 ≤50	18 (38.3%)	0.0149
	Group 2 ≥50	29 (61.7%)	
		47	
Grades of tumor	Well	7 (14.9%)	0.0107
	Moderate	25 (53.2%)	
	Poor	15 (31.9%)	

		47	
Site of tumor	Right	24 (51.07%)	0.0252
	Left	17 (36.17%)	
	Rectum	6 (12.76%)	
		47	

KRAS and BRAF comparison in group of study

A total of individuals who had been diagnosed with CRC were examined for the presence of KRAS. Positive test results for the mutation were found in 14 (48.28%) of these patients who were male, and 15 (51.72%) of these patients were female. There was a significant association between KRAS and BRAF in both female and male patients of all ages and across all age groups. This association was found in the first group of 50 patients or less than 12 years old (41.38%), and in the second group of 50 patients or more than 17 years old. Both groups included patients of all ages (58.64%).

It was discovered that 3 (10.34%) cases of CRC were well differentiated, 14 (48.28%) cases of CRC were moderately differentiated, and 12 (41.38%) cases of CRC were poorly differentiated, with a positive link being seen in both the moderately and poorly differentiated instances of CRC. The patients who had CRC were separated into groups according to the location of the tumour, which was CRC location: right colon consistent 16 (55.17%), left colon 11 (37.93%), and 2 (6.89%) in the rectum from all of the cases that had a positive relation in both the right and the left site. Right colon consistent 16 (55.17%), left colon 11 (37.93%), and from all of the cases that had a positive relation in both the right and the left Right colon consistent 16 (55.17%), left colon consistent 11 (37.93%), and among all of the instances that showed a favourable connection in both the right and the left, right colon consistent 16 (55.17%), left colon consistent 11 (37.93) The data from these investigations are presented in a condensed form in (Table 3) (Figure 1,2).

Table 3: Distributions of patients, tumours, and clinicopathologic traits according to the presence or absence of the KRAS and BRAF mutations in various persons and tumours

Gender	Status	KRAS	BRAF	P value
	Female 21	15 (51.72%)	5 (41.67%)	0.0367
	Male 26	14 (48.28%)	7 (58.33%)	0.0381
	P value	0.0722	0.039	-
Age	Group 1 ≤50 18	12 (41.38%)	6 (50%)	0.0416
	Group 2 ≥50 29	17 (58.62%)	6 (50%)	0.0435
	P value	0.0362	1	-
Morphological feature	Well 7	3 (10.34%)	1 (8.33%)	0.0677
	Moderate 25	14 (48.28%)	4 (33.33%)	0.0295
	Poor 15	12 (41.38%)	7 (58.33%)	0.0254
	P value	0.0156	0.0111	-
Site of tumor	Right24	16 (55.17%)	6 (50%)	0.0492
	Left17	11 (37.93%)	5 (41.66%)	0.0538
	Rectum 6	2 (6.89%)	1 (8.33%)	0.0723
	P value	0.01227	0.0411	-

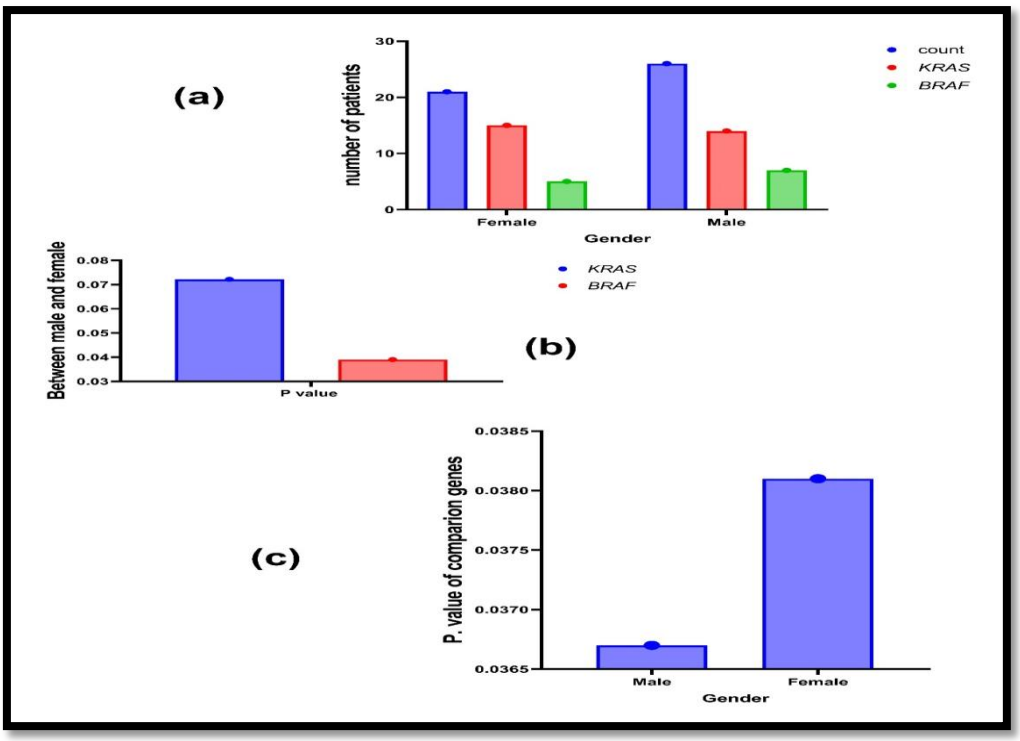

Figure 1: (a) number of patients in regarding to the gender and genes mutated, (b) comparison between p value in in regarding to the gender, (c) comparison between p value in in regarding to the genes in each group.

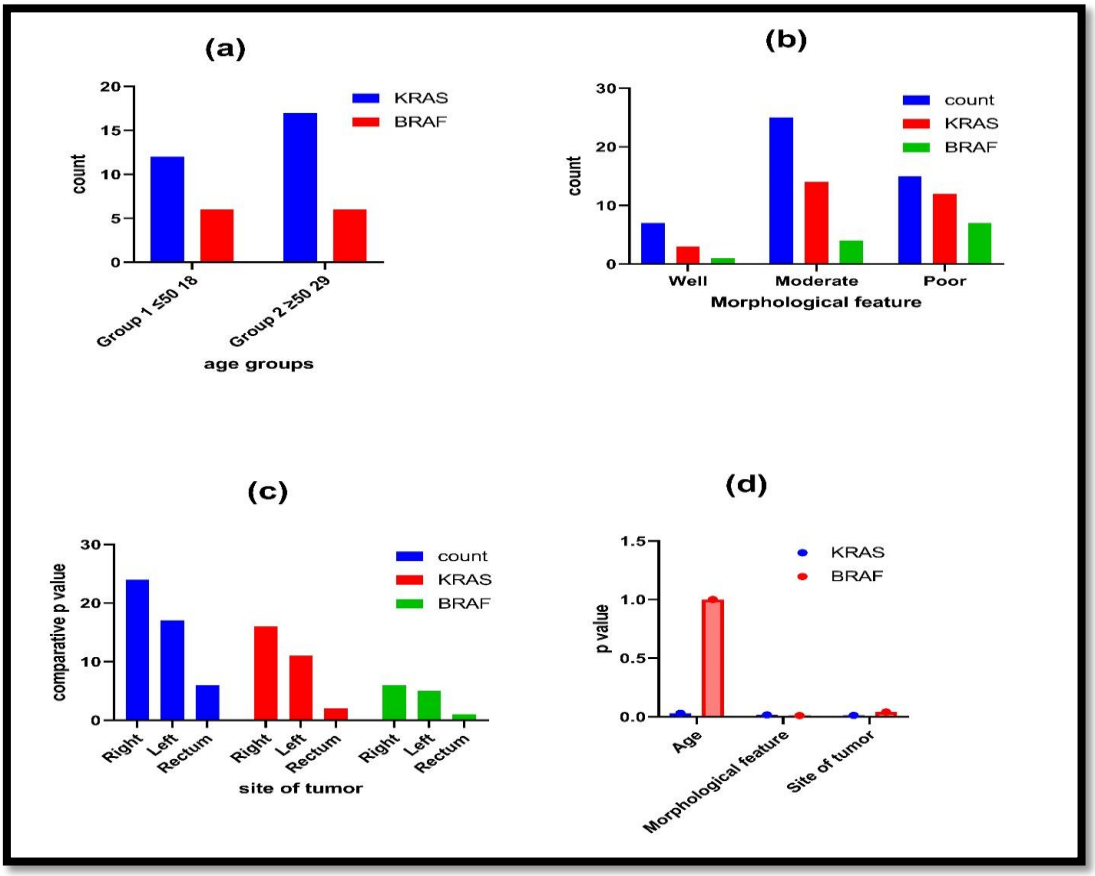

Figure 2: (a) count of patients according to the group of age in regarding to the mutation of genes, (b) count of patients according to the group of morphological features in regarding to the mutation of genes, (c) count of patients according to the sites of tumor in regarding to the mutation of genes, (d) comparative p value in gender in regarding to the genes.

DISCUSSION

In this research, a total of patients with CRC were categorised based on a variety of variables, including age, gender, the location of the tumour, and morphological feature. These factors were taken into consideration while classifying the patients. During the process of categorising the patients, these considerations were given careful attention. These findings indicate that mutations have a connection to not only the gender of the patient but also the anatomical location of the tumour. The largest prevalence of tumours with KRAS and BRAF gene mutations was seen in colon malignancies, which also had the highest incidence overall of the disease. In addition to this, the tumours that had the largest number of mutations were discovered on the right side of the large intestine, which was the location of the affected area. It was discovered that a higher percentage of female patients had KRAS mutations or while in BRAF mutations high percentage in male patients (11,26), with high percentages in both moderate and poor differentiate in both KRAS, and in poor BRAF gene mutations high percentage, which added up to a high recoded in age groups that are more than 50 years old. Moreover, it was discovered that a higher percentage of female patients had KRAS mutations or while in BRAFmutations high percentage in male patients.

According to the results of this research, a mutation in the KRAS gene was detected in 61.7% of patients, whereas a mutation in the BRAF gene was found in only 25.5% of cases. When we take into consideration the findings of a wide variety of distinct studies that concentrate on genetic mutation, we obtain the following: It was discovered that 35% of patients had the KRAS mutation, but only 14% of cancer patients had the BRAF variation in their DNA. This was a significant difference. This was a really big departure from the norm. KRAS mutations were found to be more prevalent on the right side of the cell in persons with high-grade histology, despite the fact that they were found to be more common on the left side of the cell in people with high-grade histology (P 0.001) (27,28).In spite of the fact that the data from a molecular pathological epidemiology study in a large cohort of 945 CRC patients was recoded mutations of KRAS (36.6%) and BRAF, the researchers found that there was no correlation between these mutations and the survival rates of the patients. In other words, the mutations did not affect the patients' chances of surviving the disease. Therefore, the mutations had no impact on the patients' odds of beating their cancer and surviving the disease (3.46 percent) (29).

According to the findings of the study, there is a significant correlation between clinicopathological parameters andKRAS point mutations (31.5%), BRAF gene mutations (7 percent), and clinicopathological parameters. The research also found that there is a link between clinicopathological parameters and BRAF gene mutations (30).KRAS mutations were detected in patients at a rate of 38 percent, whereas BRAF mutations were found in patients at a rate of 4.8 percent, according to certain research. When compared to men, the frequency of KRAS and BRAF gene mutations was shown to be much greater in females (55.5% vs. 41%) (p 0.005 for both). The overactivation of signalling pathways was not only identified in CRC but it was often seen in other forms of cancer as well. This phenomenon was first discovered in CRC. This is associated to the increased failure rate of DNA repair in oncogenes seen in persons with CRC (31).KRAS mutations were identified in patients at a rate of 38 percent, but BRAFmutations were found in patients at a rate of 4 percent, according to the results of some studies. Girls were found to have an incidence of KRAS and BRAF gene mutations that was nearly three times as prevalent as it was in males (55.5% vs. 41%). (p 0.005 for both). Not only was an enhanced activation of signalling pathways found in instances of CRC, but it was also often reported in cases of other types of cancer. This suggests that CRC is not the only kind of cancer in which this phenomenon occurs.CRC This is connected to the elevated failure rate of DNA repair in oncogenes that has been documented in people who have CRC (32). According to the results of our investigation, mutations were found to be associated with the gender of the patient as well as the anatomical location of the tumour. In addition to this, the tumours that included the highest number of mutations were found on the right side of the large intestine where it was located. It was discovered that a higher percentage of female patients had KRAS or BRAF mutations when compared to the percentage identified in male patients (33). A mutation in the KRAS gene was found in 38 (38.4 percent) of the primary tumours and in 36 (36.4%) of the metastases that were related with the primary tumours.This examination has the potential to be used to predict the patient's response to targeted treatment such as cetuximab and panitumumab. According to the results of this study, the identification of KRAS mutations in either the original or metastatic tumours of individuals diagnosed with CRC is likely to be concordant (34).It was discovered that the KRAS gene had been mutated in 38 of the original tumours (representing 38.4% of the total) and in 36 of the associated metastases (representing 36.4 percent of the total).An individual patient's response to a targeted therapy, such as cetuximab or panitumumab, may be anticipated with the use of this investigation. In light of the findings presented here, we have formed the hypothesis that the presence of KRAS mutations in CRC patients' primary or metastatic tumours, regardless of where the cancer originated, is highly concordant (35).In clinical practise, monoclonal antibodies like cetuximab and panitumumab are currently being utilised to selectively target EGFR in metastatic CRC. Other monoclonal antibodies include cetuximab.

The patient's reaction to the anti-EGFR therapy is reliant on the mutation status of downstream biomarkers (such as KRAS and BRAF), which have an effect on cell proliferation as well as angiogenesis and migration (36).Anti-EGFR drugs are believed to be successful of therapy for of CRC with KRAS (wild-type); however,

forty to sixty percent of patients whose tumours have wild-type KRAS do not respond to such therapy (37,38). In response to activation of the EGFR, the G protein known as KRAS undergoes a state transition that causes it to alternate between its active (KRAS-GTP) and inactive (KRAS-GDP) forms. This protein functions as a two-way switch between the surface of the cell and the signalling pathway that is farther downstream (39,40).The protein known as KRAS is a G-protein that, in response to activation of the EGFR, may transition between an active state (KRAS-GTP) and an inactive one (KRAS-GDP). This protein acts as a two-state switch between the surface of the cell and the signalling pathway that comes behind it (29,32).Among cancers that have a mutant KRAS gene (44 percent, compared to 30 percent in KRAS/BRAF wild-type tumours, P = 0.00003; 19 percent in BRAF-mutated tumours, P 0.0001) Among cancers that have a mutant KRAS gene (44 percent, compared to 30% in KRAS/BRAF wild-type tumours, P = 0.00003 (35).

It is common knowledge that aspects of a person's lifestyle, in particular the foods they eat, the cigarettes they smoke, the amount of physical exercise they get, and their ability to keep their weight under control, are responsible for the great majority of the factors that lead to cancer. It is now widely understood that epigenetics is the principal mechanism that mediates the reversible effects of dietary and lifestyle factors on the development of cancer (36). Lycopene, phytoestrogen, the polyphenols and flavonoids found in green tea, and alcohol are a few examples of other potentially bioactive dietary components. Green tea contains several beneficial compounds such as polyphenols and flavonoids. Phytoestrogens are an additional kind of potentially bioactive dietary component. In addition, there is evidence to establish a relationship between DNA methylation and both the balance of energy in the body and the level of physical activity (40).

CONCLUSION

In final analysis, the conclusion It seems, on the basis of the results of our study, that certain features are associated to mutations in KRAS and BRAF. To be more specific, a large incidence of mutant KRAS tumours and BRAFV-mutated malignancies was connected with an age of more than 50 years or older and high-grade histology. Both mutations often manifest themselves on the right side of the body; however, BRAFV600E-mutated tumours are more common in female patients than in male patients who have the same kind of cancer.

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