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The pathological significance of HRAS gene mutation in colorectal cancer patients in Erbil province

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Salar Saadi Hussain Dizayee [1*]

Abstract

Background and objective: RAS gene mutations, including Harvey RAS, are a biomarker in studying many types of cancer, including colorectal cancer. Studying the molecular and mutations of the HRAS gene help in understanding the nature of the tumor and helps to formulate a suitable plan for prevention and treatment. The aim of the present study is to identify HRAS mutations among a sample of colorectal cancer.

Methods: The sample for the present study involved 20 Formalin fixed embedded tissue of colorectal carcinoma and 10 adjacent normal tissues were obtained from Rizgary Teaching Hospital in Erbil.

Genomic DNA was isolated from 10 µm-thick paraffin-embedded tissue sections. Easy Thyroid kit to detect HRAS mutations in codons 12, 13, and 61. The HRAS mutations were based on the detection of at least 1% of tumor cells and were provided by the manufacturer of the kit. The Ct cut-off values were as follows for the different mutations: codon 12,13 Ct >35, codon 61 Ct 33.

Results: The sample of the present study involved 20 patients (60% males and 40% females) with CRC. The mean age was 54.20 years, with an SD ±19.80. The study found a single HRAS mutation in the sample study. The size of the tumor mutated by HRAS is significantly different than other tumors (P = 0.009).

Conclusion: there was an HRAS mutation in a sample of colorectal cancers and the size of the tumor was different than other tumors in the study sample. Further studies needed about the mutation of the HRAS gene.

Keywords: HRAS gene; Mutation; Colorectal cancer; Pathology.

Introduction

Colorectal cancer (CRC) is one of the most commonly occurring malignancies in the gastrointestinal tract of both sexes. It is second among women and third among men.[1-3] The death rate is the second among deaths caused by cancer. Globally, the incidence of the disease reached 1.8 million in 2018. [1,4] With continuous progress, the new cases of CRC are expected to exceed three million cases in 2040.[5] In Iraq, the burden of CRC is the seventh among cancer problems, whereas in the Iraqi Kurdistan Region the CRC is the fourth cancer burden in both sexes.[6] Anatomically, CRC usually occurs on the right side of the colon, the left side of the colon, or in the rectum. The pathogenicity, prognosis, exhibiting, distinct molecular, characteristics, and histology of cancer on the three sides are not the same.[7-9] Furthermore, the risk factors of CRC are inconsistent among anatomical diversity locations of the tumor in the colorectal.[10] Interferences between environmental and genetic factors together help to develop the tumors, including CRC.[11] Sporadic reasons have roles of 70-80% in CRC, while heredity has around 20-30% caused in the disease development.[12] The risk of CRC is associated with obesity, lack of physical activity, smoking, alcohol

[1] Department of Nursing, College of Nursing, Hawler Medical University, Erbil, Iraq.
Correspondence: salar.hussain@hmu.edu.krd

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consumption, and unhealthy eating patterns, including consumption of a high amount of salt and red meat.[13] In addition, the risk of CRC is higher among persons who have a positive family history of the disease, patients with a history of colon polyps, inflammatory bowel diseases, diabetes mellitus, cholecystectomy, gut microbiome, and aspirin exposure. Taking into consideration the roles of age, sex, and socio-economic status.[14-16] Evidence indicates that there were two causes for stimulating tumorigenesis CRC: chromosomal instability (CIN) or microsatellite instability (MIN). Sometimes it involves many proto-oncogenes, tumor suppressor genes, and also epigenetic changes in DNA.[17] With reference to the molecular installation of human cells, there are three kinds of closely linked RAS genes in human cells, called Harvey RAS (HRAS), Kirsten RAS (KRAS), and neuroblastoma (NRAS), which bear the role of encoding four highly homologous proteins. RAS proteins are known as small GTPases involved in a broad spectrum of key molecular and cellular functions, including diffusion and survival. At some time, the gain of a function missense mutation, which is commonly located at codons 12, 13, and 61, stimulates RAS proteins. These proteins can be observed in the cancer cells of humans. KRAS is the most commonly mutated, followed by NRAS and then the HRAS.[18]

The pathologist has a vital role in the diagnosis and follow-up stages of tumor growth through understanding RAS genes. Since RAS genes are among the earliest mutated genes in the majority of cancers, studying mutation patterns can help the medical team to understand how the tumor appears and develops, and also help them to identify the factors that affect the growth of the malignancy tumors. In addition, this can help to develop a plan for prevention and treatment.[19-21] Because of the modern orientation in diagnosis, treatment, and study progress of malignant tumors including CRC depending on study mutations in the genes which are responsible, the present study tries to find mutations in the region surrounding HRAS12,13,61codons and commercially available real-time-PCR formalin-fixed paraffin-embedded (FFPE) CRC tissue.

Methods

In this study, clinical characteristics of 70 CRC cases were collected by medical record survey using a standard questionnaire. The data was obtained from Riszgary Teaching Hospital in Erbil City. The demographic and clinical characteristics of patients are shown in supplementary table1. 20 archival formalin-fixed paraffin embedded tissues of colorectal carcinoma case selected for molecular analysis by RT-PCR. Genomic DNA was isolated from 10 µm-thick paraffin- embedded tissue sections.

Sections were deparafinated twice for 30 min in xylene, re-dehydrated in 100,80,60,40% ethanol for 10 sec. ,then added 200 ml Qiagen tissue lysis buffer (Qiaamp DNA extraction kit) red transfer to eppendorf tubes and incubated with 40 µl protein-kinase and incubated overnight at 37℃ add 20 ml protease K incubated for 1-2 h at 55℃ after a total pro-k incubation DNA isolation proceeded as in the manufacture protocol.[22]

The DNA concentration was determined at 260 nm using the Nano Nano drop spectrometer (thermos-fisher-USA). Polymerase chain reaction PCR-based assay (Easy Thyroid kit) for identifying HRAS mutation, located in codons 12,13,61. In real-time or quantitative PCR, the amount of product is mentioned during the reaction. The number of amplification cycles required to obtain a certain amount of PCR product is registered as the threshold cycle (Ct) (20). A mutation is present when the Ct (which is the Ct of the mutation-specific PCR minus the Ct of a PCR of an endogenous control gene) is below a mutation-specific threshold. The Ct values that were used as a cut–off level for the presence of an HRAS mutation were

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based on the detection of at least 1% tumor cells and were provided by the manufacturer of the kit. The Ct cut-off values were as follows for the different mutations: codon 12,13 Ct >35, codon 61 Ct > 33. The assay was validated for analytical and diagnostic use and performed according to the manufacturer's instructions on a Real-Time PCR System [Roter- gene Q (Qiagen)].[23] The Ethical Committee at the College of Nursing, Hawler Medical University, has approved the study.

Statistical analysis

The Statistical Package for Social Science (SPSS, version 26) was used for data entry and analysis. Descriptive statistical analysis (including frequency, percentage, mean, standard deviation, range, and ratio) was used to describe the data; and inferential statistical analysis was used to determine the association between variables by using Fisher's exact tests. The *P*-value is considered statistically significant if it's 0.05 which rejects the null hypothesis.

Results

The mean age of the study sample was 52.23 years, with SD ±16.23, the range of age was 7 to 82 years. The distribution of the sample according to sex was 64.3 % males and 35.7% females (Table 1). The male to female ratio is 1.8:1.

Table 1 shows that the highest proportion 21.4% of tumors were located on the proximal, followed by the rectum and ileum, 17.1% and 17.1% respectively. Most (94.3%) tumors were adenocarcinoma type. Concerning the size of tumors, the majority (72.9.9%) of tumors were less than 4 cm and 18.6% were more than 4 cm. Regarding vascular invasion, the table shows that 70.0% of tumors were positive for vascular invasion. The same table showed that 72.9% of tumors were positive regarding the nodal state. In reference to pathological stages, Table 1 shows that 61.4% of tumors are in grade III and 30.0% in grade II.

Figure 1 shows that there was positive mutation in HRAS gene by 5% in sample of present study

Table 2 shows that only a single case of positive mutation in the HRAS gene; was in the sigmoid location, the size was more than 4 cm; and it is an adenocarcinoma type of tumor, the tumor in the third stage of growth. The table shows, that the case was reached in the vascular invasion phase and the tumor is the nodal state.

Table 2 shows that there was a significant difference between the size of the tumor and the positive HRAS mutation (*P* = 0.009).

The table shows that the case is between the age group 32 to 48 years, and 8 (40%) of patients who were negative regarding gene mutation were in the age group 66 and more, there were no significant differences in age groups and HRAS mutation (*P* = 0.368); and the case was female on another hand 12 (60%) of patients who were negative about gene mutation were males, with no significant differences regarding sex and gene mutation (*P* = 0.209).

Table 2 shows that there were no significant differences in terms of tumor location and HRAS gene mutation (*P* = 0.947). The same table shows that 18 (90%) of patients who were negative in the mutation of the HRAS gene were adenocarcinoma type of tumor, there were no significant differences regarding the type of tumor and HRAS mutation (*P* = 0.814).

Table 2 shows that 9 (45%) of patients who were negative about gene mutation their tumors were in stage II of growth, and there were no significant differences in the stage of tumor growth and HRAS mutation (*P* = 0.293).

Considering the grade of tumor and nodal state, results show that there were no significant differences between the tumor mutated to HRAS gene and other tumors in the study sample (*P* = 0.117 and 0.117 respectively).

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Table 1 Age, sex, clinical and histopathological features of colorectal cancer patients

Items	No.	(%)
Age categories		
≤21	1	(1.4)
22-31	7	(10.0)
32-41	12	(17.1)
42-51	9	(12.9)
52-61	21	(30.0)
62-71	13	(18.6)
72-81	6	(8.6)
≥82	1	(1.4)
Sex		
Male	45	(64.3)
Female	25	(35.7)
Location		
Rectum	12	(17.1)
Ileum	12	(17.1)
Sigmoid	6	(8.6)
large intestine	1	(1.4)
Proximal	15	(21.4)
Distal	6	(8.6)
Transverse colon	4	(5.7)
Hemicolectomy	1	(1.4)
Caecal cancer	1	(1.4)
Mid rectal	2	(2.9)
Sigmoid annular	1	(1.4)
Caecalasending colon mass	1	(1.4)
Descendind colon	2	(2.9)
Upper rectal cancer	1	(1.4)
Lower rectal	1	(1.4)
Assending Colon	1	(1.4)
Colorectal	1	(1.4)
CA Sigmoid	2	(2.9)
Size per cm		
<4	51	(72.9)
4	6	(8.5)
>4	13	(18.6)
Tumor type		
Adenocarcinoma	66	(94.3)
Mucinous carcinoma	2	(2.9)
Hyperchromatic	1	(1.4)
Tubulo-villous adenoma	1	(1.4)
Grade		
First	6	(8.6)
Second	21	(30.0)
Third	43	(61.4)
Vascular invasion		
Positive	49	(70.0)
Negative	21	(30.0)
Nodal state		
Positive	51	(72.9)
Negative	19	(27.1)
Total	70	(100.0)

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Table 2 HRAS mutation and its association with age, sex, clinical, and histopathological features

Item	Mutation				*P*-Value
	Positive		Negative		
	No.	(%)	No.	(%)	
Age					
< 32	0	(0.0)	3	(100.0)	
32-48	1	(20.0)	4	(80.0)	**0.368**
49-65	0	(0.0)	4	(100.0)	
≥66	0	(0.0)	8	(100.0)	
Sex					
Male	0	(0.0)	12	(100.0)	**0.209**
Female	1	(12.5)	7	(87.5)	
Location					
Ileum	0	(0.0)	2	(100.0)	
Sigmoid	1	(33.3)	2	(66.7)	
Proximal	0	(0.0)	1	(100.0)	
Hemicolectomy	0	(0.0)	1	(100.0)	
Cecal cancer	0	(0.0)	1	(100.0)	
Mid rectal	0	(0.0)	2	(100.0)	
Sigmoid annular	0	(0.0)	1	(100.0)	
Cecal ascending colon mass	0	(0.0)	1	(100.0)	**0.947**
Descending colon	0	(0.0)	2	(100.0)	
Upper rectal cancer	0	(0.0)	1	(100.0)	
Lower rectal	0	(0.0)	1	(100.0)	
Ascending Colon	0	(0.0)	1	(100.0)	
Colorectal	0	(0.0)	1	(100.0)	
CA Sigmoid	0	(0.0)	2	(100.0)	
Size/cm					
< 4	0	(0.0)	17	(100.0)	
4	0	(0.0)	1	(100.0)	**0.009**
> 4	1	(50.0)	1	(50.0)	
Tumor type					
Adenocarcinoma	1	(5.3)	18	(94.7)	**0.814**
Tubulo-villous adenoma	0	(0.0)	1	(100.0)	
Grade					
First	0	(0.0)	5	(100.0)	**0.293**
Second	0	(0.0)	9	(100.0)	
Third	1	(16.7)	5	(83.3)	
Vascular Invasion					
Positive	1	(16.7)	5	(83.3)	**0.117**
Negative	0	(0.0)	14	(100.0)	
Nodal State					
Positive	1	(16.7)	5	(83.3)	**0.117**
Negative	0	(0.0)	14	(100.0)	
Total	1	(100.0)	19	(100.0)	

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Discussion

The present study shows that nearly half of the CRC cases were recorded among patients aged 52 to 71. The mean age was 52.23 years, with SD ±16.23, and the ratio of males exceeded that of females. A study was carried out by Homady et al. in Najaf Province, Iraq. Results show that 80% of patients with CRC were aged 40 or more, and the mean age of patients was 51.44% with SD ±16.67.[24] A study was carried out in Taiwan. Results reported that the proportion of CRC in the elderly has increased over the past 20 years. The study recommended the necessity of doing healthy screening for individuals who have aged more than 75 years.[25] Results of a study done by White and his colleagues indicated that the overall incidence of CRC is higher in men, with an earlier age distribution, however, important sex differences exist in the anatomical sites of tumors. The researchers in that study believe that it is crucial to consider gender as a key factor in the diagnosis and treatment of colon cancer.[26]

The present study shows that more than one-fifth of tumors were proximal located, most of them adenocarcinoma, at 61.4% in stage III, the highest percentage of tumors positive in vascular invasion and also the highest percentage of tumors positive concerning the nodal state. The study of Ahmed et al, sample involved 120 CRC patients. Results reported that the highest percentage (55.8%) of patients presented with colonic site tumors (among them 90% of tumors were right colon tumors spread to local and distant sites), followed by recto-sigmoid (10.8%). Adenocarcinoma (AD) was diagnosed in the majority (87.5%). The highest percentage of patients were categorized with moderately differentiated carcinoma representing 63.3%, followed by well-differentiated and poorly differentiated carcinomas which constituting 29.2% and 7.5%, respectively. [27]

The present study found that there was a single case (5%) of HRAS mutation in a sample consisting of 20 patients with CRC. In a study by Ye et al. In China, a sample involved 1190 patients with CRC diagnosed between May 1998 and December 2018 and received clinical genetic testing. The test of the OncoCarta Panel has been used to test 19 common mutations of oncogenes. Results reported that there were only 11(0.9%) H-RAS mutations in the sample.[28] The study sample of Chang et al. involved 1,519 CRC patients. Results indicated that 1.7% of HRAS mutations were reported in that sample.[29] A study by Maffeis et al.

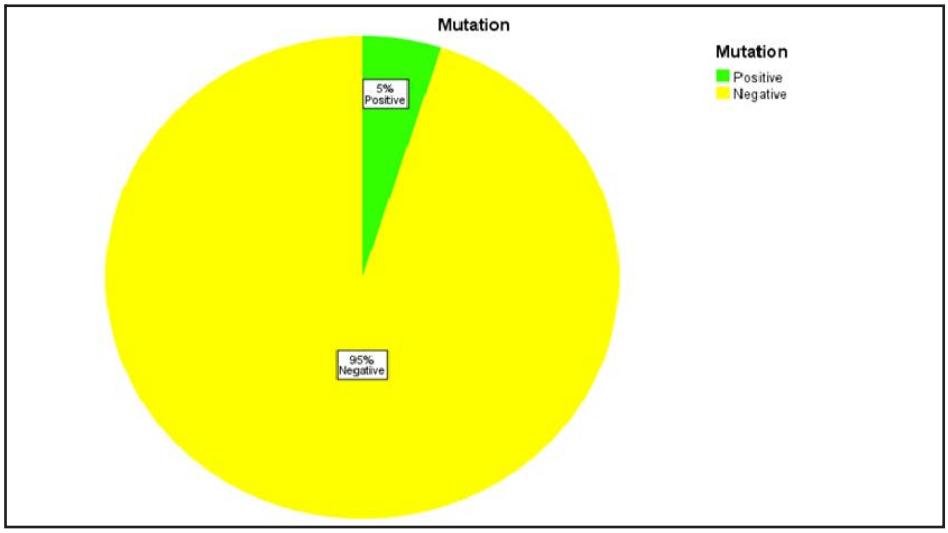

Figure 1 HRAS mutation in colorectal cancer

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indicated only rare cases of HRAS mutated had been reported among a sample of CRC patients.[30] While Fernández-Medarde reported that no active HRAS mutation had been detected in a sample of CRC patients.[31] A cohort study by Serebriskii et al., involved 13336 CRC patients. The results showed the mutational profiles of KRAS, NRAS, and less common HRAS in CRC tumors, comparing the frequency of specific mutations based on the age of diagnosis, Microsatellite Instability status, and colon versus rectum sub-site.[32]

The present study shows that the CRC case that was exposed to HRAS mutation was female, and she is in the age group 32 -48 years. A study by Feng et al, indicated that HRAS expression was higher in female patients than males, and in cancers with distant metastasis compared to those with non-distant metastasis.[33] A study was carried out by Abudabous et al, in Libya. The sample involved 34 CRC patients 19 males and 15 females. The age range is 24- 87 years. Results show that HRAS mutations were not detected in any of the patients in the study group, that study reported that the KRAS codon 12/13 mutations were present in 38.2% (13/34) of the patients.[34] The tumor of the patient with HRAS mutation in the present study is sigmoid location. The HRAS mutations were not associated with any of the clinical-pathological features in the study of Chang et al.[29] The tumor size in the case that occurred by HRAS mutation was more than four centimeters, with significant differences between the size of the mutated tumor and other tumors in the study sample. Because HRAS mutation in CRC was rare according to many studies,[28,30,31] H-RAS may play an essential role in the carcinogenesis of CRC,[29] there are not enough studies had been done about clinical and pathological features of CRC developed by HRAS mutation, and further studies are needed to carrying out to clarifying molecular mutation of HRAS, in addition, more studies need for identifying clinical and pathological features of CRC tumors that developed by HRAS mutation.

The HRAS muted tumor in the present study is adenocarcinoma. Globally, more than 90% of CRC cases are adenocarcinoma.[35] The tumor in the present study is positive regarding the nodal state and vascular invasion. A univariate logistic analysis study involving 600 patients was carried out by Xu et al. about lymph node metastasis. Results revealed that colon cancer is significantly correlated (P <0.05) with tumor size, grading, stage, preoperative carcinoembryonic antigen (CEA) level, and perineural invasion.[36]

Conclusion

The present study concluded that 48.6% of CRC patients were in age 52-71 years, male more than female. The study concluded more than one-third (21.4%) of tumors were in proximal location, most tumors were adenocarcinoma, and sizes of 18.6% of tumors were more than 4 cm. The study concluded that there was a case of CRC developed by HRAS mutation, and the size of that tumor was significantly higher than the size of other tumors in the study sample. More studies need to be done about the mutation of the HRAS gene in colorectal carcinoma.

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Competing interests

The author declares that she has no competing interests.

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