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Molecular Analysis of KRAS Mutation Associated with Colorectal Cancer in Iraqi Patients

[1]Rehab Subhi Ramadhan, [1]Aola Sabah Ali and [2]Rebah Najah Algafari

[1]College of Biotechnology, Al-Nahrain University, Iraq
[2]Biotechnology Research Center, Al-Nahrain University, Iraq

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Corresponding Author:
Rehab Subhi Ramadhan
College of Biotechnology, Al-Nahrain University, Iraq
Email: rehabrebah2004@yahoo.com

Abstract: The role of KRAS gene was investigated in manifestation of colorectal cancer in Iraqi patients. A total 40 blood samples were collected during October 2016 to January 2017 from AL-Amal Hospital in Baghdad and 20 blood samples from healthy subjects served as control. Blood samples were collected from subjects of 40-70 years old for both patients and control. The study found that age group 61-70 years old were more susceptible to colorectal cancer with ratio of 40% more than younger individuals involved in this study with higher frequency in males with 75.5% than females who show 49.5% ($p>0.01$) when both compared to the same gender. DNA extracted from positive cancer samples and control were subjected to specific PCR amplification using 10 specifically designed primers for this study to amplify KRAS gene exons. DNA sequencing for the resulting amplicons showed the presence of significant genetic change that included substitution and insertion, causing 15% frame shift and 85% missense changes at positions 5920, consequently led to sever disruption in KRAS function.

Keywords: KRAS, Oncogenes, Colon Cancer, DNA Aberration

Introduction

Colorectal cancer represents (15%) of worldwide malignancies and is the third cause of cancer in men (10%) of total and the second cause of cancer in women after breast cancer (9.4%) of total (Center *et al*., 2009). Colorectal cancer is a complex disease influenced by both genetic and environmental factors. Lifestyle and environmental risk factors include, for instance, smoking, diet and physical inactivity (Giovannucci, 2002; Botteri *et al*., 2008). Interestingly, physical inactivity has been estimated to cause up to (10%) of the burden of Colorectal cancer (Lee *et al*., 2012). Environmental and lifestyle factors partly explain the high rate of colorectal cancer observed in the Western world. In addition, an increased risk for CRC has also been reported for individuals with Inflammatory Bowel Disease (IBD) (Dyson and Rutter, 2012). The development of colorectal cancer is a multistep process characterized by the accumulation of genetic alterations (Arteaga, 2002). Along the progression from normal colonic epithelial cells, small adenoma, advanced adenoma and finally to carcinoma, the KRAS oncogene mutation has a role in a significant proportion of CRCs. The KRAS gene encodes a 21-kDa small protein that is activated transiently as a response to extracellular stimuli or signals such as growth factors, cytokines and hormones via cell surface receptors (Malumbres and Barbacid, 2003). The gene is located on the short arm of chromosome 12 (12p). It is mutated in (40-50%) of sporadic cases of CRC. KRAS has been reported to be mutated in about (30%) of colorectal adenomas and (30%) to (50%) of CRCs. Kirsten Rat Sarcoma viral oncogene homolog (KRAS) is an oncogene that encodes a small GTPase transduction protein called KRAS (Castagnola and Giaretti, 2005). The point mutations are seen in codon 12 in most cases but also at codon 13 and 61. It has been shown that KRAS mutations in metastatic disease are a predictor of resistance to Cetuximab (anti-EGFR) therapy and, furthermore, they are associated with a worse prognosis of disease (Karapetis *et al*., 2008).

The aim of study was the detection the single nucleotide polymorphism in KRAS gene associated with incidence of colorectal cancer.

Materials and Methods

Collection of Blood Samples: The blood samples were collected at Al-Amal hospital from 40 patients after they diagnosed with colorectal cancer using bio marker (CA19-9). Their ages ranged between (40-70) years as the majority of individuals attending the hospital for treatment. A volume of 5 mL of peripheral blood was collected by vein puncture and divided into two tubes, 1 mL to EDTA tube and store at -20°C for DNA extraction. The 4 mL was transferred to a plain tube, for a biomarker test (CA19-9).

DNA extraction from blood: The gSYNC™ DNA Extraction Kit from Geneaid (Taiwan) was used for this purpose as instructed by the manufacturer. The extraction procedure mainly depends upon spin column technique which gave DNA purity of 1.8-2 and concentration of 80-120 ng/sample.

Primers used for DNA amplification: KRAS exons were amplified using the following primers designed in this study.

Primer name		Sequences 3`---- 5`	Product size
1	F	GTCTCCCTGTGTCAGACTGC	433
	R	AATGTCTTGGCACACCACCA	
2	F	TCCCTGTGTCAGACTGCTCT	313
	R	AGGACCACCACAGAGTGAGA	
3	F	AGGGACTAGGGCAGTTTGGA	386
	R	CACCTCACCATGCCATCTCA	
4	F	TCTCCCTGTGTCAGACTGCT	642
	R	ACCTCACCATGCCATCTCAC	
5	F	TGGTGGTGTGCCAAGACATT	480
	R	CCCAAGACTGGCACTGAAGA	
6	F	CTTCCACATGCCCCATGACT	370
	R	ACTGTTACCAGGAGCAGTCC	
7	F	TCTTGCCTCCCTACCTTCCA	385
	R	TACTGTTACCAGGAGCAGTCC	
8	F	CCTCCCTACCTTCCACATGC	378
	R	CTGTTACCAGGAGCAGTCCTA	
9	F	ACTGTTGTCACCATTGCACA	734
	R	TGTGCATGTTTCAGTTTACACTAT	
10	F	GTTTGAAGTGCCTGTTTGGGA	324
	R	CACAAAAGAAAGCACAATGTACAAA	

DNA Amplification Programs and PCR Conditions

Because of the different melting temperatures for each set of primer, the following conditions were used for optimum results:

Primers 1, 7 and 8

Thermal cycler protocol	No. of cycle	Temperature -time
Initial Denaturation	1 cycle	94°C for 5 min
Denaturation	35cycle	94°C for 1 min
Annealing		59°C for 1 min
Extension		72°C for 1 min
Final extension	1 cycle	72°C for 10 min

Primers 2, 3, 4, 5 and 6

Thermal cycler protocol	No. of cycle	Temperature -time
Initial Denaturation	1 cycle	94°C for 5 min
Denaturation	35cycle	94°C for 1 min
Annealing		56°C for 1 min
Extension		72°C for 1 min
Final Extension	1 cycle	72°C for 10 min

Primers 9 and 10

Thermal cycler protocol	No. of cycle	Temperature-time
Initial Denaturation	1 cycle	94°C for 5 min
Denaturation	35cycle	94°C for 1 min
Annealing		53°C for 1 min
Extension		72°C for 1 min
Final extension	1 cycle	72°C for 10 min

Electrophoresis Conditions

The resulting PCR products were subjected to electrophoresis using 2% agarose at 10 v/cm field strength for 1 h and photographed using Biorad gel documentation system.

Statistical analYsis

The Statistical Analysis System-SAS (2012) program was used to signify the relation among age, gender and colorectal cancer manifestation.

Results

In this study, forty blood samples were collected from patients suffering from colorectal cancer and 20 healthy (control), with age ranging from 40 to 70 years. Chi-square tests was used to establish the relation between age and gender. Results are shown in Table 1.

Colorectal cancer was found to be more frequent in male than female as shown in Table 2.

Molecular Analysis of KRAS

Ten primers were designed to amplify all KRAS exons to be used for sequencing and subsequent analysis. Results showed that there is a significant change in DNA sequence among patients and as shown in Fig. 1.

Further details of genetic changes are listed in Table 3.

In almost all patients subjected to DNA analysis of KRAS gene, genetic change included missense (83 SNPs) mutation with less frequency of frame-shift (2 SNPs) resulting in deformed protein and non functional enzyme.

Table 1: Distribution of patients according to age group

Age group (years)	Number of patients	Percentage (%)
40-50	10	25
51-60	14	35
61-70	16	40
Total	40	100
Chi–square value (x^2)	-	9.261**

**(P<0.01)

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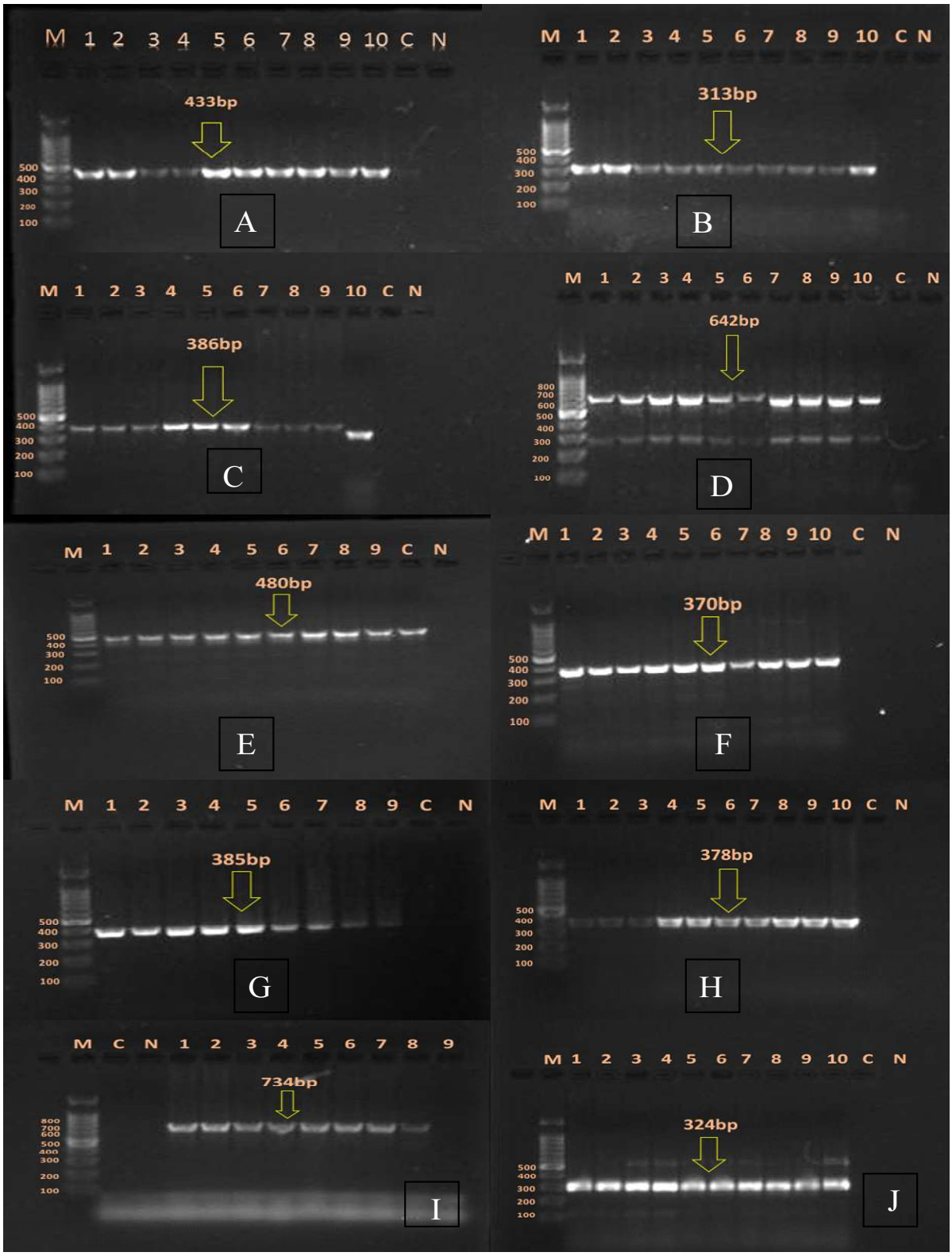

Fig. 1:KRAS specific amplification using primers designed in this study. Designations A, B, C, D, E, F, G, H, I and J represent primers,1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. M is 100 bp DNA marker, whereas C and N are control negative and normal DNA respectively. Patients are designated with numbers from 1-10

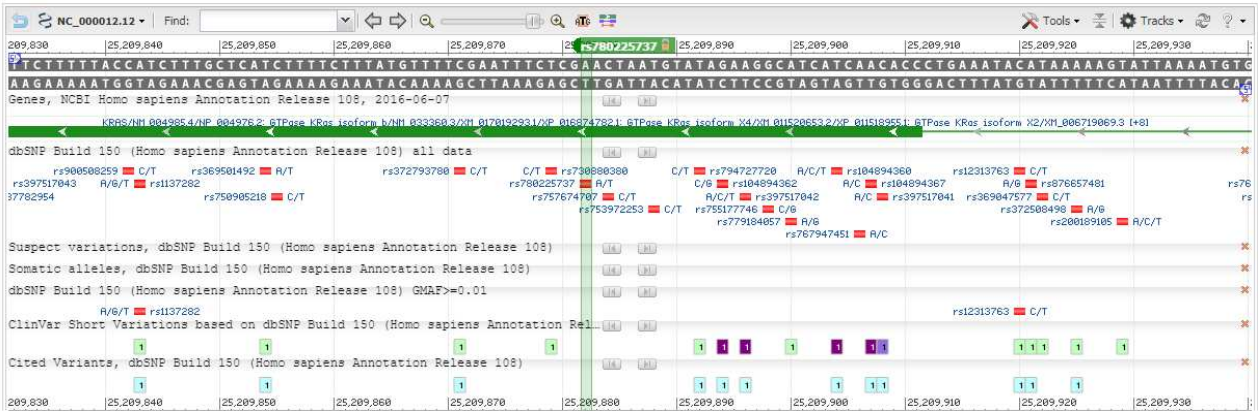

Fig. 2: Mapping the genetic change in KRAS gene. The figure shows Graphical distribution of KRAS SNPs in the sequence obtained from colorectal cancer patients. The figure also elaborates amino acid change resulted from the SNP which resulted in pathogenic effect (red dots) and purple squares, whereas light blue squares represent non effective SNPs. The figure was generated using NCBI analysis tool (https://www.ncbi.nlm.nih.gov/protein/NP_203524.1?report=graph&v=1:63&content=5&m=13)

Table 2: Distribution of patients according to gender

Gender	Number of patients	Percentage (%)	Chi-square value (x^2)
Male	23	57.50	10.974**
Female	17	42.50	10.052**
Total	40	100%	9.261**

**(P<0.01)

Table 3: SNP linked to Gene KRAS Via Contig Annotation. The table was generated using The SNP GeneView for human variation depending on the sequence obtained in this study. The tool used for analysis is available at https://www.ncbi.nlm.nih.gov/ analysis tools

Chr. position	mRNA pos	dbSNP rs# cluster id	Hetero-zygosity	Clinical Significance	Function	dbSNP allele	Protein residue	Codon pos	Amino acid pos
25209808	746	rs775000854	0.000		Missense	A	Tyr [Y]	2	185
					Contig reference	G	Cys [C]	2	185
25209822	730	rs749587181	0.000			-		1	180
25209822	732	rs762532538	0.000		Missense	C	Asn [N]	3	180
					Contig reference	G	Lys [K]	3	180
25209826	728	rs763736188	0.000		Missense	G	Arg [R]	2	179
					Contig reference	A	Lys [K]	2	179
25209828	725	rs587782954	N.D.	Uncertain significance		GAA	Lys [K]	2	180
25209828	726	rs751233524	N.D.		Synonymous	G	Lys [K]	3	178
					Contig reference	A	Lys [K]	3	178
25209829	723	rs397517043	0.001	Uncertain significance		-		3	180
25209842	712	rs900508259	N.D.		Missense	A	Ser [S]	1	174
					Contig reference	G	Gly [G]	1	174
25209843	711	rs1137282	0.316	Likely benign	Synonymous	C	Asp [D]	3	173
					Contig reference	T	Asp [D]	3	173
25209854	700	rs369501492	0.000	other	Missense	T	Leu [L]	1	170
					Contig reference	A	Met [M]	1	170
25209855	699	rs750905218	0.000		Synonymous	A	Lys [K]	3	169
					Contig reference	G	Lys [K]	3	169
25209871	683	rs372793780	0.000	Uncertain significance	Missense	A	Gln [Q]	2	164
					Contig reference	G	Arg [R]	2	164
25209879	675	rs730880380	N.D.	Benign	Synonymous	G	Arg [R]	3	161
					Contig reference	A	Arg [R]	3	161
25209882	672	rs780225737	0.000		Synonymous	A	Val [V]	3	160
					Contig reference	T	Val [V]	3	160
25209884	670	rs757674707	N.D.		Missense	A	Ile [I]	1	160
					Contig reference	G	Val [V]	1	160
25209888	666	rs753972253	0.000		Synonymous	G	Thr [T]	3	158
					Contig reference	A	Thr [T]	3	158
25209892	662	rs794727720	N.D.	Uncertain significance	Missense	G	Cys [C]	2	157
					Contig reference	A	Tyr [Y]	2	157

Table 3: Continuo

25209894	660	rs104894362	N.D.	Pathogenic	Missense	G	Leu [L]	3	156
					Contig reference	C	Phe [F]	3	156
25209896	658	rs397517042	N.D.	Pathogenic	Missense	A	Ile [I]	1	156
					Missense	G	Val [V]	1	156
					Contig reference	T	Phe [F]	1	156
25209898	656	rs755177746	0.000		Missense	G	Gly [G]	2	155
					Contig reference	C	Ala [A]	2	155
25209900	654	rs779184057	0.000	Uncertain significance	Synonymous	C	Asp [D]	3	154
					Contig reference	T	Asp [D]	3	154
25209904	650	rs104894360	N.D.	Pathogenic	Missense	G	Gly [G]	2	153
					Missense	T	Val [V]	2	153
					Contig reference	A	Asp [D]	2	153
25209906	648	rs767947451	0.000		Synonymous	G	Val [V]	3	152
					Contig reference	T	Val [V]	3	152
25209907	647	rs104894367	N.D.	Pathogenic	Missense	G	Gly [G]	2	152
					Contig reference	T	Val [V]	2	152
25209908	646	rs397517041	N.D.	Likely pathogenic	Missense	T	Phe [F]	1	152
					Contig reference	G	Val [V]	1	152
25225620	636	rs777364720	N.D.		Synonymous	G	Thr [T]	3	148
					Contig reference	A	Thr [T]	3	148
25225625	631	rs387907206	N.D.	Pathogenic	Missense	G	Glu [E]	1	147
					Contig reference	A	Lys [K]	1	147
25225628	628	rs121913527	N.D.	Uncertain significance	Missense	A	Thr [T]	1	146
					Contig reference	G	Ala [A]	1	146
25225632	624	rs766109434	0.000		Synonymous	G	Thr [T]	3	144
					Contig reference	A	Thr [T]	3	144
25225641	615	rs138669124	0.000		Missense	A	Leu [L]	3	141
					Contig reference	T	Phe [F]	3	141
25225651	605	rs754870563	0.000		Missense	A	Glu [E]	2	138
					Contig reference	G	Gly [G]	2	138
25225652	604	rs778702415	0.000		Missense	A	Arg [R]	1	138
					Contig reference	G	Gly [G]	1	138
25225653	603	rs752731198	0.000		Synonymous	C	Tyr [Y]	3	137
					Contig reference	T	Tyr [Y]	3	137
25225657	599	rs757816355	0.000		Missense	A	Asn [N]	2	136
					Contig reference	G	Ser [S]	2	136
25225663	593	rs373500216	0.000		Missense	G	Gly [G]	2	134
					Contig reference	C	Ala [A]	2	134
25225675	581	rs730880473	0.000	Uncertain significance	Missense	T	Val [V]	2	130
					Contig reference	C	Ala [A]	2	130
25225681	575	rs746609817	0.000		Missense	G	Arg [R]	2	128
					Contig reference	A	Lys [K]	2	128
25225683	573	rs770720889	0.000		Synonymous	G	Thr [T]	3	127
					Contig reference	A	Thr [T]	3	127
25225684	572	rs781634879	0.000		Missense	G	Arg [R]	2	127
					Contig reference	C	Thr [T]	2	127
25225694	562	rs575569675	0.000		Missense	T	Ser [S]	1	124
					Contig reference	A	Thr [T]	1	124
25225709	547	rs730880471	N.D.	Likely pathogenic	Missense	A	Asn [N]	1	119
					Contig reference	G	Asp [D]	1	119
25225713	543	rs770248150	0.000		Missense	C	Asn [N]	3	117
					Contig reference	A	Lys [K]	3	117
25225717	539	rs202247812	N.D.	untested	Missense	G	Ser [S]	2	116
					Contig reference	A	Asn [N]	2	116
25225730	526	rs775836436	0.000		Missense	A	Ile [I]	1	112
					Contig reference	G	Val [V]	1	112
25225742	514	rs763553461	0.000		Missense	T	Tyr [Y]	1	108
					Contig reference	G	Asp [D]	1	108
25227234	482	rs727503106	N.D.	Likely pathogenic	Missense	A	Lys [K]	2	97
					Contig reference	G	Arg [R]	2	97
25227260	456	rs370920665	0.000	Uncertain significance	Synonymous	G	Lys [K]	3	88
					Contig reference	A	Lys [K]	3	88
25227262	454	rs953088090	N.D.		Missense	G	Glu [E]	1	88
					Contig reference	A	Lys [K]	1	88
25227263	453	rs397517038	N.D.	Uncertain significance	Frame shift	-	Asn [N]	3	88
					Contig reference	T	Lys [K]	3	88
25227275	441	rs751117590	0.000		Synonymous	T	Ala [A]	3	83
					Contig reference	C	Ala [A]	3	83

Table 3: Continuo

25227288	428	rs868857258	N.D.		Missense	C	Pro [P]	2	79
					Contig reference	T	Leu [L]	2	79
25227294	422	rs756890312	0.000		Missense	C	Ala [A]	2	77
					Contig reference	G	Gly [G]	2	77
25227296	420	rs727503107	N.D.	Uncertain significance	Synonymous	A	Glu [E]	3	76
					Contig reference	G	Glu [E]	3	76
25227300	416	rs780974222	0.000		Missense	C	Ala [A]	2	75
					Contig reference	G	Gly [G]	2	75
25227302	414	rs745580373	0.000		Synonymous	C	Thr [T]	3	74
					Contig reference	T	Thr [T]	3	74
25227304	412	rs770020203	0.000		Missense	G	Ala [A]	1	74
					Contig reference	A	Thr [T]	1	74
25227305	411	rs104886027	N.D.	untested	Synonymous	A	Arg [R]	3	73
					Contig reference	G	Arg [R]	3	73
25227308	408	rs104886028	N.D.	untested	Missense	A	Ile [I]	3	72
					Contig reference	G	Met [M]	3	72
25227310	406	rs727504662	N.D.	Pathogenic	Missense	T	Leu [L]	1	72
					Contig reference	A	Met [M]	1	72
25227313	403	rs387907205	N.D.	Likely pathogenic	Missense	C	His [H]	1	71
					Missense	C	His [H]	1	71
					Missense	G	Asp [D]	1	71
					Missense	G	Asp [D]	1	71
					Contig reference	T	Tyr [Y]	1	71
					Contig reference	T	Tyr [Y]	1	71
25227314	402	rs780492744	0.000		Synonymous	A	Gln [Q]	3	70
					Contig reference	G	Gln [Q]	3	70
25227326	390	rs200229810	0.000	Uncertain significance	Synonymous	G	Ala [A]	3	66
					Contig reference	A	Ala [A]	3	66
25227335	379	rs730880469	N.D.	Likely pathogenic		-		1	63
25227341	375	rs17851045	N.D.	Pathogenic	Missense	C	His [H]	3	61
					Missense	T	His [H]	3	61
					Contig reference	A	Gln [Q]	3	61
25227342	374	rs121913240	N.D.	Pathogenic	Missense	C	Pro [P]	2	61
					Missense	G	Arg [R]	2	61
					Missense	T	Leu [L]	2	61
					Contig reference	A	Gln [Q]	2	61
25227343	373	rs121913238	N.D.	Pathogenic	Missense	A	Lys [K]	1	61
					Missense	G	Glu [E]	1	61
					Contig reference	C	Gln [Q]	1	61
25227344	372	rs397517037	N.D.	Uncertain significance	Synonymous	A	Gly [G]	3	60
					Contig reference	T	Gly [G]	3	60
25227345	371	rs727503108	N.D.	Pathogenic	Missense	T	Val [V]	2	60
					Contig reference	G	Gly [G]	2	60
25227346	370	rs104894359	N.D.	Pathogenic	Missense	A	Ser [S]	1	60
					Missense	C	Arg [R]	1	60
					Contig reference	G	Gly [G]	1	60
25227348	368	rs104886029	N.D.	untested	Missense	T	Val [V]	2	59
					Contig reference	C	Ala [A]	2	59
25227349	367	rs121913528	N.D.	Likely pathogenic	Missense	A	Thr [T]	1	59
					Missense	A	Thr [T]	1	59
					Missense	T	Ser [S]	1	59
					Missense	T	Ser [S]	1	59
					Contig reference	G	Ala [A]	1	59
					Contig reference	G	Ala [A]	1	59
25227351	365	rs104894364	N.D.	Pathogenic	Missense	T	Ile [I]	2	58
					Contig reference	C	Thr [T]	2	58
25227356	360	rs958790148	N.D.		Synonymous	T	Leu [L]	3	56
					Contig reference	C	Leu [L]	3	56
25227374	342	rs774909024	0.000		Synonymous	G	Thr [T]	3	50
					Contig reference	C	Thr [T]	3	50
25227376	340	rs730880470	N.D.	Uncertain significance	Missense	T	Ser [S]	1	50
					Contig reference	A	Thr [T]	1	50
25227386	330	rs904755552	N.D.		Missense	G	Met [M]	3	46
					Contig reference	T	Ile [I]	3	46
25245277	300	rs727503109	N.D.	Likely pathogenic	Missense	G	Met [M]	3	36
					Contig reference	A	Ile [I]	3	36
25245284	293	rs104894366	N.D.	Pathogenic	Missense	G	Arg [R]	2	34
					Missense	T	Leu [L]	2	34
					Contig reference	C	Pro [P]	2	34

Table 3: Continuo

25245292	285	rs377354475	0.000	Uncertain significance	Synonymous	G	Glu [E]	3	31
					Contig reference	A	Glu [E]	3	31
25245295	282	rs113623140	0.001	Benign	Synonymous	T	Asp [D]	3	30
					Contig reference	C	Asp [D]	3	30
25245309	268	rs794727277	N.D.	Uncertain significance	Missense	T	Tyr [Y]	1	26
					Contig reference	A	Asn [N]	1	26
25245312	264	rs754767487	0.000		Frame shift	T	Ser [S]	3	25
					Contig reference	-	Gln [Q]	3	25
25245317	260	rs730880472	N.D.	Likely pathogenic	Missense	G	Arg [R]	2	23
					Contig reference	T	Leu [L]	2	23
25245320	257	rs727503110	N.D.	Pathogenic	Missense	G	Arg [R]	2	22
					Missense	T	Leu [L]	2	22
					Contig reference	A	Gln [Q]	2	22
25245321	256	rs121913236	N.D.		Missense	A	Lys [K]	1	22
					Contig reference	C	Gln [Q]	1	22
25245325	252	rs747140926	0.000		Synonymous	A	Thr [T]	3	20
					Contig reference	G	Thr [T]	3	20
25245328	249	rs121913538	N.D.	Uncertain significance	Missense	C	Phe [F]	3	19
					Missense	T	Phe [F]	3	19
					Contig reference	G	Leu [L]	3	19
25245330	247	rs771188508	0.000		Synonymous	C	Leu [L]	1	19
					Contig reference	T	Leu [L]	1	19
25245334	243	rs776785730	0.000		Synonymous	C	Ser [S]	3	17
					Contig reference	T	Ser [S]	3	17
25245345	232	rs104894365	0.000	Pathogenic	Missense	A	Ile [I]	1	14
					Contig reference	G	Val [V]	1	14
25245346	231	rs397517040	0.000	Likely pathogenic	Synonymous	A	Gly [G]	3	13
					Synonymous	T	Gly [G]	3	13
					Contig reference	C	Gly [G]	3	13
25245347	230	rs112445441	N.D.	Pathogenic	Missense	A	Asp [D]	2	13
					Contig reference	G	Gly [G]	2	13
25245348	229	rs121913535	N.D.	Pathogenic	Missense	A	Ser [S]	1	13
					Missense	C	Arg [R]	1	13
					Missense	T	Cys [C]	1	13
					Contig reference	G	Gly [G]	1	13
25245350	227	rs121913529	0.000	Pathogenic	Missense	A	Asp [D]	2	12
					Contig reference	G	Gly [G]	2	12
25245351	226	rs121913530	0.000	Pathogenic	Missense	A	Ser [S]	1	12
					Missense	C	Arg [R]	1	12
					Missense	T	Cys [C]	1	12
					Contig reference	G	Gly [G]	1	12
25245352	225	rs397517039	N.D.	Likely benign	Synonymous	C	Ala [A]	3	11
					Contig reference	T	Ala [A]	3	11
25245355	221	rs606231202	N.D.	Pathogenic		TGG	Gly [G]	2	10
25245361	216	rs147406419	0.001	other	Synonymous	G	Val [V]	3	8
					Contig reference	A	Val [V]	3	8
25245370	207	rs104894361	N.D.	Pathogenic	Missense	T	Asn [N]	3	5
					Contig reference	A	Lys [K]	3	5
25245372	205	rs193929331	N.D.	Pathogenic	Missense	G	Glu [E]	1	5
					Contig reference	A	Lys [K]	1	5

It's noteworthy to mention that there is (31 SNPs) are synonymous change representing the redundancy of the nucleotide that may be considered as a genetic imprint of Iraqi KRAS gene.

Detailed distribution of SNPs are represented in Fig. 2.

Discussion

Colorectal cancer poses a great threat of malicious diseases that may appear in human. Most of such cancer cases may be attributed to the diet of people, age, gender and accumulation of genetic changes during aging which may trigger malignant tumors especially in families with such history (Brenner *et al*., 2007). Patients consent for this study were divided according to the gender and age. Most cases of colorectal cancer were found in male at elderly age which may be attributed to physiological factors, stress and type of diet, whereas less colorectal cancer were diagnosed in female. No significant malignant tumors were found in younger individuals. For rectal cancer, male and female rates are similar at younger ages, but male rates increasingly predominate at older ages. The frequency of ras mutations to differ significantly with respect to age and sex of patients and location of tumour. This might indicate that there is an association between activation of K-ras and the differences in incidence of colorectal cancer (Breivik *et al*., 1994).

Table 4: The clinical significance of SNPs found in this study

No.	Clinical significance	No. of SNPs
1.	Likely benign	2
2.	Uncertain significance	16
3.	Benign	2
4.	Likely pathogenic	9
5.	Pathogenic	22

In addition, degeneration of immune system, diminish of repair system and apoptosis at elderly subjects made them more candidate to colorectal cancer (Levin *et al*., 2008).

Most of literatures (Bader and Ismail, 2014; Hilmi *et al*., 2015; Oleg *et al*., 2015) mentioned that mutation at codon 12 and 13 are more frequent in elderly men than women, that significantly trigger malignant tumor at the colon.

Literatures regarding colorectal cancer in Arabian countries (Wafa and Maher, 2012; Abulkair *et al*., 2016) studied KRAS mutation at codon 12 and 13 only, but non of these articles detailed the full spectrum of SNPs and their clinical significant as whole.

The study showed that exon 2 at position 5920 located on chromosome 6 may play a vital role in controlling cell cycle and was the main target for genetic changes. Most of missense mutations and frame shift mutations mutations were found at this exon that led consequently to significant change in the resulting protein rendering it with less or no function.

The clinical significance of our finding is listed in Table 4.

Conclusion

Colorectal cancer is a malicious disease that appear mostly in male than female and was more abundant in elderly subjects. This disease is highly affected by diet and gender and mostly appeared in subjects with family history for malignant tumors. Most of genetic changes were at chromosome 6 position 5920 that included in missense mutation at high rate, frame shift at lower rate. Such accumulative changes led to produce non-functional protein, which stopped apoptosis process in malignant cells causing the tumors to appear in patients.

Ethics Approval and Consent to Participate

This study did not include any human subjects and was performed on animals postmortem.

Consent for Publication

This work did not include any personal, written information, pictures and videos to any person.

Availability of Data and Material

All data and materials used in this study are available and stored at Biotechnology Research Center, Baghdad University and Institut für Virusdiagnostic Reference Laboratory in Germany.

Competing Interests

This work was conducted without conflict of interest among authors or any other research group in others institutes.

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Authors' Contributions

All authors contributed to this work according to their major in designing, following, interpreting and data analysis.

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