Clinicopathological features of Egyptian colorectal cancer patients regarding somatic genetic mutations especially in *KRAS* gene and microsatellite instability status: a pilot study

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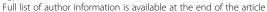
Abstract

Background: Colorectal cancer (CRC) is the third most common cause of cancer-related deaths which contributes to a significant public health problem worldwide with 1.8 million new cases and almost 861,000 deaths in 2018 according to the World Health Organization. It exhibits 7.4% of all diagnosed cancer cases in the region of the Middle East and North Africa. Molecular changes that happen in CRCs are chromosomal instability, microsatellite instability (MSI), and CpG island methylator phenotype. The human *RAS* family (*KRAS*, *NRAS*, and *HRAS*) is the most frequently mutated oncogenes in human cancer appearing in 45% of colon cancers. Determining MSI status across CRCs offers the opportunity to identify patients who are likely to respond to targeted therapies such as anti-PD-1. Therefore, a method to efficiently determine MSI status for every cancer patient is needed.

Results: *KRAS* mutations were detected in 31.6% of CRC patients, namely in older patients ($p = 0.003$). Codons 12 and 13 constituted 5/6 (83.3%) and 1/6 (16.7%) of all *KRAS* mutations, respectively. We found three mutations G12D, G12C, and G13D which occur as a result of substitution at c.35G>A, c.34G>T, and c.38G>A and have been detected in 4/6 (66.6%), 1/6 (16.7%), and 1/6 (16.7%) patients, respectively. Eleven (57.9%) patients had microsatellite instability-high (MSI-H) CRC. A higher percentage of MSI-H CRC was detected in female patients ($p = 0.048$). Eight patients had both MSI-H CRC and wild *KRAS* mutation with no statistical significance was found between MSI status and *KRAS* mutation in these studied patients.

Conclusion: In conclusion, considering that *KRAS* mutations confer resistance to EGFR inhibitors, patients who have CRC with *KRAS* mutation could receive more tailored management by defining MSI status. MSI-high patients have enhanced responsiveness to anti-PD-1 therapies. Thus, the question arises as to whether it is worth investigating this association in the routine clinical setting or not. Further studies with a larger number of patients are needed to assess the impact of MSI status on Egyptian CRC care.

Keywords: Colorectal cancer, Targeted NGS, *KRAS* mutation, MSI

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Background

Colorectal cancer (CRC) is the third most common cause of cancer-related deaths which contributes to a significant public health problem worldwide with 1.8 million new cases and almost 861,000 deaths in 2018 according to the World Health Organization. It exhibits 7.4% of all diagnosed cancer cases in the region of the Middle East and North Africa [1]. Globally, the highest CRC incidence rates are in Australia, New Zealand, Europe, and North America, and the lowest rates are detected in Africa and south Central Asia [2]. Despite the fact that the overall incidence of CRC in teenagers and young adults is low, it has seen an expansion over the past decades. This is caused by a lack of routine screening in the young population and lifestyle issues such as obesity and dietary factors [3]. Molecular changes that happen in CRC might be classified into three principals groups: (1) chromosomal instability (CIN), (2) microsatellite instability (MSI), and (3) CpG island methylator phenotype (CIMP) [4]. The human *RAS* family (*KRAS*, *NRAS*, and *HRAS*) is the most frequently mutated oncogenes in human cancer appearing in 90% of pancreatic, 35% of lung, and in 45% of colon cancers. Mutation in Kirsten rat sarcoma 2 viral oncogene homolog (*KRAS*) is the most important widely used molecular test in the CRC metastatic settings due to the presence of this mutation which can predict a lack of benefit from epithelial growth factor receptor (*EGFR*)-targeted antibodies. These *KRAS* mutations are highly frequent in the codons 12, 13, 59, 61, and 146 [1, 5]. B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) gene mutations are detected in 15% of all cancers, and the V600E mutation is detected in 10% of all CRCs. However, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene is implicated in the PI3K/Akt pathway and is mutated in ~ 10–20% of CRCs where ~ 80% mutations are found in two hotspot areas in exons 9 and 20. Also, the patients with mutations in *BRAF* and *PIK3CA* have shown a negative response to *EGFR* inhibitor therapy [1]. Phosphatase and tensin homolog (*PTEN*) is located in 10q23.31, and its main function is to block the PI3K pathway by dephosphorylating phosphatidylinositol (PI) 3,4,5-triphosphate into PI-4,5-bisphosphate thus opposing PI3K function. *PTEN* is found mutated in 3.86% of CRC patients. Finally, tumor protein p53 (*TP53*) is located in 17p13.1. Somatic mutations in the *TP53* gene are the most common genetic changes found in human cancer occurring in about 50% of CRCs with the most frequently observed mutations between exons 5 and 8 at codons 175, 213, 245, 248, 273, and 282 [1, 4]. Microsatellites, also known as short tandem repeats (STRs), are small (1–6 bp) repeating stretches of DNA scattered all through the whole genome (both in coding and non-coding regions)

and account roughly for 3% of the human genome. Because of their repeated structure, microsatellites are subjected to a high mutation rate. Microsatellite instability (MSI) in tumor DNA is defined as the presence of alternate sized repetitive DNA sequences that are absent in the corresponding germline DNA. MSI is a molecular phenotype due to a defective DNA mismatch repair system [6]. DNA mismatch repair (MMR) system corrects erroneous insertion, deletion, and base-base mismatches generated during DNA replication and recombination that have escaped the proofreading process [6]. Tumors are classified as MMR-deficient (dMMR) if they have somatic or germline mutations. There are several methods to detect MSI, the Bethesda panel of microsatellite markers comprising 2 mononucleotide repeat markers (BAT25 and BAT26) and three dinucleotide repeat markers (D2S123, D5S346, and D17S250). If at least two or more microsatellite markers are mutated, the tumor is considered MSI-high (MSI-H); if just one is mutated, the tumor is defined as MSI-low (MSI-L); and if none of the examined loci exhibits instability, the tumor will be considered microsatellite stable (MSS). A few years later, it was discovered that mononucleotide markers have a better sensitivity and specificity as the dinucleotide markers have a polymorphic nature. So, the uses of panels containing more mononucleotide markers have been expanded due to their higher sensitivity and specificity in the diagnosis of MSI in CRCs [7]. MSI has been associated with improved prognosis as MSI-high cancers are a genetically defined subset of cancers with the potential for enhanced responsiveness to anti-PD-1 therapies. Determining MSI status across cancer types offers the opportunity to identify patients who are likely to respond while avoiding unnecessary toxicities for patients identified as unlikely to respond [8]. Therefore, a method to efficiently determine MSI status for every cancer patient is needed.

Study objectives

Our objective was to demonstrate the clinicopathologic features of Egyptian CRC patients together with identification and correlation with somatic genetic changes and the MSI status in those patients.

Methods

Study population and sample collection

The present study included 19 newly diagnosed fresh surgical tumor specimens from 19 CRC patients attending to the outpatient clinic of our department. All patients were subjected to surgical resection where the surgeon removed the part of the colon that contains the tumor mass, along with a margin of normal tissue on either side of the mass. After gross examination, macro dissection was done. Then, hematoxylin and eosin (H&E) staining was performed on serial sections. The tissue area representing the

"tumor" which contains the highest numbers of cancer cells was identified and "normal" which contains no malignant tissue identified and separated. Healthy control tissue was included in our study for the interpretation of MSI results.

Patients were selected according to the following criteria: Inclusion criteria:

– Age group: adulthood CRC patients
– Gender: males and females
– Pathologically proved colorectal adenocarcinoma.
– Stages II, III, and IV
– Newly diagnosed cases attending the outpatient clinic

Exclusion criteria:

– Patients with double malignancy
– Known HIV-positive or AIDS-related illness
– Women who are pregnant or breastfeeding

Our study was exploratory in nature and performed in the molecular lab of our institution, in the period from March 2018 to February 2019. The study was approved by our department Institutional Review Board (IRB)-3-2018. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments (GCP guidelines) or comparable ethical standards.

DNA extraction
Genomic DNA was extracted from tissues with the QIAamp DNA Mini kit (Qiagen, USA) as reported by the manufacturer and was eluted in 60 μl volume. The extracted DNA samples were measured utilizing the Qubit dsDNA High Sensitivity (HS) assay kit (Life Technologies, Fisher Scientific, USA). A quantitative PCR (qPCR) reaction was performed to conclude the amplifiability of the extracted gDNA samples and to predict assay success. All samples with ΔCq value below or equal to 2 can be selected for further use.

Library preparation
Libraries were prepared using TruSight Tumor 15 kit according to the manufacturer's protocol (Illumina, Inc., San Diego, CA). TruSight Tumor 15 uses next-generation sequencing (NGS) to assess 15 of the most commonly mutated significant genes in CRC in a single assay: AKT serine/threonine kinase 1 (*AKT1*), G protein subunit alpha 11 (*GNA11*), *NRAS*, *BRAF*, G protein subunit alpha q (*GNAQ*), platelet-derived growth factor receptor alpha (*PDGFRA*), *EGFR*, KIT proto-oncogene receptor tyrosine kinase (*KIT*), *PIK3CA*, erb-b2 receptor tyrosine kinase 2

(*ERBB2*), *KRAS*, ret. proto-oncogene (*RET*), forkhead box L2 (*FOXL2*), MET proto-oncogene, receptor tyrosine kinase (*MET*), and *TP53*. Library quality was checked out by 2100 Bioanalyzer utilizing the Bioanalyzer DNA 1000 reagents and chips (Agilent Technologies, Santa Clara, CA). Successful library amplification was estimated when the expected PCR product size is ~ 350 bp. Before sequencing, the libraries together with PhiX control library were normalized following the manufacturer's protocol, and equal volumes were pooled to constitute the terminal sequencing library. The TruSight Tumor 15 sequencing panel achieves limits of detection of 5% variant allele frequency across 250 amplicons with 93.5% of bases covered at ≥ 500× [9].

Sequencing and data analysis
Sequencing was accomplished utilizing MiSeqDx device (Illumina) with a 2 × 151 bp read length and a total time of 27 h which involve cluster generation, sequencing, and base calling on the MiSeqDx system. Specifications based on Illumina PhiX control library support cluster densities between 1200 and 1400 k/mm^2 clusters passing filter for v3 chemistry. The quality scores > 80% bases higher than Q30 at 2 × 151 bp. Image processing and VCF file generation were further analyzed; we then annotated the variants using VariantStudio™ software version 3. This software determined the numerical identifier for each variant in the COSMIC database, if the genomic variant position overlapped a variant recorded in COSMIC. The COSMIC ID linked to the COSMIC page associated with the identifier to determine information on whether that variant has been detected before in any cancer patient or a novel one. Synonymous variants and non-coding regions were filtered out. Mutations with low depth, which indicate ≤ 50× depths, were filtered out. And mutations with ≤5% variant allele frequency were filtered out. Variant quality which is one parameter of the variant call format (VCF) < 80% was filtered out.

MSI analysis
We evaluated the MSI status using three mononucleotide repeat markers (BAT25, BAT26, and NR27). The kit was purchased from Qiagen Co., with primer sequences as follows:

BAT-25 (product size, 114 bp): 5′-CTCGCCTCCAAG AATGTAAGT-3′; 5′-CTATGGCTCTAAAATGCTCTG TTC-3′

BAT-26 (product size, 122 bp): 5′-TGACTACTTT TGACTTCAGCC-3′; 5′-AACCATTCAACATTTTTA ACCC-3′

NR-27 (product size, 89 bp): 5′-AACCATGCTTGCAA ACCACT-3′; 5′-CGATAATACTAGCAATGACC-3′.

Amplification reactions (20 μl) were prepared with 10–100 ng extracted DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.25 mM dNTP, 0.5 μM of each primer, and 0.5 units Platinum® Taq DNA Polymerase (Invitrogen). Reactions were subjected to PCR amplification: initial incubation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 54 °C for 45 s, and 72 °C for 60 s, and a final incubation at 72 °C for 10 min. PCR products were analyzed on the Bioanalyzer 2100 system. The results were evaluated by comparing every tumor DNA to DNA from the healthy control tissue. Peaks present in the tumor tissue which were not found in the normal tissue indicated instability of a marker. Samples with no altered markers were classified as MSS. Samples with only one altered microsatellite marker were classified as MSI-L. Samples with ≥ 2 altered markers were classified as MSI-H [10].

Statistical analysis

Statistical analysis was done using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. A chi-square test was used to examine the relation between the qualitative variables. For not normally distributed quantitative data, a comparison between the two groups was done using the Mann-Whitney test (non-parametric t test). All tests were two-tailed. A p value ≤ 0.05 was considered significant.

Results

Patients' characteristics

Nineteen CRC patients were incorporated in our study, and their clinicopathological features were shown in Table 1. The median age was 40 years (range, 17–72 years), and 8 (42.1%) patients were < 40 years old. CRC was more frequently observed in females (63.2%) than in males (36.8%). Regarding tumor location, 52.6% of the patients had tumor mass in the proximal colon, 31.6% in the distal colon, and only 15.8% in the rectum. Primary tumors were higher in the right colon (52.6%) than in the left side (47.4%). The size of the tumor was observed to be < 5 cm in 14 (73.7%) patients. As regards the TNM staging system, a higher percentage of patients were noted to have tumor stage III (52.6%), followed by stage II (42.1%) and stage IV (5.3%). The N0, N1, and N2 stages of lymph node invasion had percentages of 52.6%, 42.1%, and 5.3%, respectively. The invasion depth of the tumor in the layers of the colon/rectum showed that T3 represented 94.7% of all cases, followed by 5.3% in T4 with no case observed in T1or T2. Finally, the presence of distant metastasis was noticed in only one patient (5.3%).

Table 1 Clinicopathological features of the studied CRC patients

CRC patients' characteristics	Number (%)
Gender	
Males	7/19 (36.8%)
Females	12/19 (63.2%)
Age (years)	
Range	17-72
Mean ± SD	40.42 ± 17.54
Median	40
≥ 40	11/19 (57.9)
< 40	8/19 (42.1%)
Tumor location	
Proximal colon	10/19 (52.6%)
Distal colon	6/19 (31.6%)
Rectum	3/19 (15.8%)
Tumor site	
Left	9/19 (47.4%)
Right	10/19 (52.6%)
Size of tumor (cm)	
< 5	14/19 (73.7%)
≥ 5	5/19 (26.3%)
Tumor stage	
I	0
II	8/19 (42.1%)
III	10/19 (52.6%)
IV	1/19 (5.3%)
No. of nodes	
N0 (0)	10/19 (52.6%)
N1(1–3)	8/19 (42.1%)
N2 (> 3)	1/19 (5.3%)
Depth of invasion	
T1	0
T2	0
T3	18/19 (94.7%)
T4	1/19 (5.3%)
Distant metastasis	
Yes	1/19 (5.3%)
No	18/19 (94.7%)

Somatic mutations results

We determined somatic mutations in 15 genes using targeted NGS on 19 CRC patients, and the mutation list in all patients was described in Table 2. KRAS mutations were detected in 6/19 (31.6%) patients. Codons 12 and 13 constituted 5/6 (83.3%) and 1/6 (16.7%) of all *KRAS* mutations, respectively. These mutations were substitutions of glycine with aspartate or cyseine on codon 12 and substitution of glycine with aspartate on codon 13. These 3 mutations

Table 2 Patients' mutational data

No.	Gene	Variant	Consequence	Chr	Exons	dbSNP ID	HGVSc	HGVSp	In silico predictions		COSMIC ID	MSI status
									Sift	PolyPhen		
1	PIK3CA	G>A	Missense	3	10/21	rs104885999	c.1571G>A	p.(Arg524Lys)	Tolerated (0.95)	Benign	COSM53245	MSI-L
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
2	KRAS	G>A	Missense	12	2/6	rs112445441	c.38G>A	p.(Gly13Asp)	Deleterious (0.04)	Possibly damaging (0.505)	COSM1140132	MSI-L
											COSM532	
	TP53	C>T	Missense	12	8/11	rs28934574	c.844C>T	p.(Arg282Trp)	Deleterious	Probably damaging (0.995)	COSM99925	
											COSM10704	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
	PIK3CA	C>A	Missense	3	10/21	rs121913286	c.1636C>A	p.(Gln546Lys)	Deleterious (0.03)	Probably damaging (0.95)	COSM766	
											COSM255876	
3	TP53	G>A	Missense	17	7/11	rs11540652	c.743G>A	p.(Arg248Gln)	Deleterious	Probably damaging (1)	COSM10662	MSI-L
											COSM99602	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
	PIK3CA	C>G	Missense	3	10/21	rs121913285	c.1616C>G	p.(Pro539Arg)	Deleterious	Possibly damaging (0.795)	COSM446010	
											COSM759	
4	TP53	C>G	Missense	17	5/11	rs138729528	c.523C>G	p.(Arg175Gly)	Deleterious	Probably damaging (0.985)	COSM707883	MSI-H
											COSM707882	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
5	ERBB2	G>A	Missense	17	21/27	–	c.2524G>A	p.(Val842Ile)	Deleterious	Probably damaging (1)	COSM14065	MSI-H
											COSM5034439	
6	Wild											MSI-L
7	TP53	C>T	Stop gained	17	6/11	rs397516435	c.586C>T	p.(Arg196Ter)	–	–	COSM99668	MSI-L
											COSM99667	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
8	KRAS	G>A	Missense	12	2/6	rs121913529	c.35G>A	p.(Gly12Asp)	Deleterious	Benign (0.36)	COSM521	MSI-H
											COSM1135366	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
9	KIT	A>C	Missense	4	10/21	rs3822214	c.1621A>C	p.(Met541Leu)	Tolerated (0.37)	Benign	COSM28026	MSI-H
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
	ERBB2	A>G	Missense	17	17/27	rs1136201	c.1963A>G	p.(Ile655Val)	Tolerated (0.75)	Benign (0.045)	COSM4000121	
											COSM5019265	
10	TP53	C>T	Missense	17	7/11	rs121912651	c.742C>T	p.(Arg248Trp)	Deleterious	Probably damaging (1)	COSM10656	MSI-H
											COSM3388183	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061	
											COSM3766193	
	TP53	C>T	Stop gained	17	10/11	rs730882029	c.1024C>T	p.(Arg342Ter)	–	–	COSM99721	
											COSM11073	

Table 2 Patients' mutational data *(Continued)*

No.	Gene	Variant	Consequence	Chr	Exons	dbSNP ID	HGVSc	HGVSp	In silico predictions		COSMIC ID	MSI status
									Sift	PolyPhen		
11	KRAS	G>A	Missense	12	2/6	rs121913529	c.35G>A	p.(Gly12Asp)	Deleterious	Benign (0.36)	COSM521 COSM1135366	MSI-L
	TP53	G>A	Missense	17	5/11	rs28934578	c.524G>A	p.(Arg175His)	Tolerated (0.11)	Benign (0.31)	COSM10648 COSM99914	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	
12	KRAS	G>A	Missense	12	2/6	rs121913529	c.35G>A	p.(Gly12Asp)	Deleterious	Benign (0.36)	COSM521 COSM1135366	MSI-H
	TP53	C>T	Missense	17	8/11	rs28934574	c.844C>T	p.(Arg282Trp)	Deleterious	Probably damaging(0.995)	COSM99925 COSM10704	
	TP53	T>C	Missense	17	6/11	rs760043106	c.584 T>C	p.(Ile195Thr)	Deleterious	Probably damaging (1)	COSM11089 COSM116922	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	
13	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	MSI-H
14	KRAS	G>T	Missense	12	2/6	rs121913530	c.34G>T	p.(Gly12Cys)	Deleterious (0.04)	Probably damaging (0.995)	COSM1140136 COSM516	MSI-H
	TP53	C>A	Missense	17	8/11	–	c.832C>A	p.(Pro278Thr)	Deleterious	Probably damaging (1)	COSM43697 COSM368635	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	
15	KRAS	G>A	Missense	12	2/6	rs121913529	c.35G>A	p.(Gly12Asp)	Deleterious	Benign (0.36)	COSM521 COSM1135366	MSI-L
	PIK3CA	G>A	Missense	3	10/21	rs104885999	c.1571G>A	p.(Arg524Lys)	Tolerated (0.95)	Benign	COSM53245	
	KIT	G>A	Missense	4	10/21	rs55792975	c.1594G>A	p.(Val532Ile)	Tolerated (0.06)	Benign (0.03)	–	
	TP53	T>G	Missense	17	7/11	–	c.712 T>G	p.(Cys238Gly)	Deleterious	Probably damaging (1)	COSM131450 COSM46336	
	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	
16	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	MSI-H
17	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	MSI-H
18	PIK3CA	G >A	Missense	3	10/21	rs104885999	c.1571G>A	p.(Arg524Lys)	Tolerated (0.95)	benign	COSM53245	MSI-L
19	TP53	C>G	Missense	17	4/11	rs1042522	c.215C>G	p.(Pro72Arg)	Tolerated (0.57)	Benign (0.145)	COSM250061 COSM3766193	MSI-H

G12D, G12C, and G13D occur as a result of substitution at c.35G>A, c.34G>T, and c.38G>A and have been detected in 4/6 (66.6%), 1/6 (16.7%), and 1/6 (16.7%) patients, respectively. A higher percentage of *KRAS* mutations was detected in female patients (5/6, 83.3%) than in male patients (1/6, 16.7%) with no statistically significant difference between the two groups, *p* value =

0.22. Statistical comparison between CRC patients with wild and mutant *KRAS* mutation revealed no statistically significant difference between the two groups as regards tumor site, stage, no. of lymph nodes, depth of invasion, and distant metastasis except for their age with *p* value = 0.003 where mutant *KRAS* was detected in older CRC patients.

MSI status results

Among the examined 19 CRC patients, 11 (57.9%) patients had MSI-H CRC. A higher percentage of MSI-H CRC was detected in female patients (9/11, 81.8%) than in male patients (2/11, 18.2%) with a statistically significant difference between the two groups, *p* value = 0.048. Statistical comparison between CRC patients with MSI-H and MSI-L/MSS revealed no statistically significant difference between the two groups as regards their age, tumor site, stage, no. of lymph nodes, depth of invasion, and distant metastasis. Out of 11 MSI-H CRC patients, only 3 patients had mutant *KRAS*. All mutations were on codon 12 with 2 patients having a substitution of glycine with aspartate and 1 patient having a substitution of glycine with cyseine.

Eight patients had both MSI-H CRC and wild *KRAS* mutation with no statistically significant correlation between MSI status and *KRAS* mutation in our CRC patients. Correlation of patients' clinicopathological features with KRAS mutations and MSI status was described in Table 3.

Discussion

Colorectal cancer care is becoming increasingly relied on tumor biomarkers to diagnose, select optimal therapy, and anticipate prognosis for each patient. Also, the detection of somatic mutations within patient tumors plays an important role in the risk stratification of CRC patients. Previous studies have shown that *KRAS* and MSI were prognostic biomarkers for CRC with a

Table 3 Correlation of patients' clinicopathological features with KRAS mutational status and MSI status

CRC patients' characteristics	KRAS wild (N = 13/19)	KRAS mutant (N = 6/19)	*p* value	MSI-H (N = 11/19)	MSI-L/MSS (N = 8/19)	*p* value
Gender						
Males	6	1	0.22	2	5	0.048*
Females	7	5		9	3	
Age (years)	33 ± 14.37	56.5 ± 12.57	0.003*	43.09 ± 19.28	36.75 ± 15.26	0.45
Tumor location						
Proximal colon	8	2		6	4	
Distal colon	2	4	0.18	2	4	0.06
Rectum	3	0		3	0	
Tumor site						
Left	5	4	0.25	5	4	0.85
Right	8	2		6	4	
Size of tumor (cm)						
< 5	9	5	0.52	8	6	0.91
≥ 5	4	1		3	2	
Tumor stage						
II	5	3		5	3	0.59
III	7	3	0.74	5	5	
IV	1	0		1	0	
No. of nodes						
N0 (0)	7	3		6	4	0.85
N1 (1–3)	5	3		4	4	
N2 (> 3)	1	0		1	0	
			0.88			
Depth of invasion						
T3	12	6	0.49	10	8	0.38
T4	1	0		1	0	
Distant metastasis						
Yes	1	0	0.49	1	0	0.38
No	12	6		10	8	
MSI-H (N = 11/19)	8	3		–	–	–
MSI-L/MSS (N = 8/19)	5	3	0.63			

*Significant at *p* ≤ 0.05

significant association of both with the clinicopathological features in those patients [11]. So, the present study aimed to demonstrate the somatic genetic changes in 15 genes especially *KRAS* mutation using targeted next-generation sequencing and to evaluate MSI status with their correlation to clinicopathological features of Egyptian CRC patients. In our study, *KRAS* mutation was detected in 31.6% of CRC patients. This is comparatively less than the average level of *KRAS* mutations (35–45%) found in CRCs, the mutation proportions observed in Serbia, Turkey, Saudi Arabia, and Egypt (32–35.7%), and the mutation proportions observed in Slovenia, Iraq, and Spain (46.2–48%), and higher than the mutation proportions observed in Morocco and Greece and the previously reported proportion in India (23.5–29.3%) [12–14]. *KRAS* mutation was insignificantly higher in female CRC patients, and this is in agreement with our previous study [1]. Other studies found that *KRAS* mutation was more likely to be noted in male subjects ($p = 0.010$) [15]. However, in other reports, an even distribution of *KRAS* mutations has been described in both genders [16]. There was no significant difference in the clinicopathological features between *KRAS* mutant and *KRAS* wild patients except for patient age where *KRAS* mutation was detected in older individuals with p value = 0.003. Other studies found no significant correlation with gender, ethnicity, age, tumor differentiation, histological type, and the presence of distant metastases. However, they found in patients with metastatic disease, *KRAS* mutations were more common in individuals older than 65 years ($p = 0.035$) [16]. Regarding MSI status, 57.9% of our patients had MSI-H CRC which was reported as a factor associated with improved prognosis [17]. Previous studies found that MSI-H sporadic CRCs are the most often (about 70–95%) caused by alteration of the MLH1 gene via somatic promoter hypermethylation [18]. In other Egyptian studies, MSI-H was found in 37% and 30.8% of their patients [17, 19]. This difference may be attributed due to different detective methods to some extent. MSI-H CRC was significantly higher in female patients with p value = 0.048. This is in agreement with Tsai et al. who reported that the female patients revealed significantly a MSI-high status (14.0 vs. 8.3%, OR 1.800, $p = 0.001$) than male patients [20]. However, other reports did not find a significant difference between MSI-H versus MSS/MSI-L regarding gender [21, 22]. Statistical comparison between CRC patients with MSI-H and MSI-L/MSS revealed no statistically significant difference between the two groups as regards their age, tumor site, tumor location, TNM stage, no. of lymph nodes, depth of invasion, and distant metastasis. However, MSI-H CRCs have been reported to demonstrate a more frequent association with proximal tumor location, poorly differentiated, mucin-containing histology, and

advanced staging [23]. Another study revealed that proximal location (right side) was selected as predictors of MS-H CRC with $p = 0.005$ [24]. In our study, only three patients had a mass in the rectum, and they all had MS-H CRC and wild *KRAS*. However, MSI-H cancers are rarely located in the rectum in Western populations, but fully half of Egyptian MSI-H cases were at that site [19]. Finally, there was no statistically significant correlation between MSI status with *KRAS* mutation in our CRC patients. However, Ashktorab et al. revealed that the incidence of *KRAS* mutations is inversely correlated with MSI-H status in CRCs [23]. Again, Liu et al. revealed that *KRAS*-mutated CRCs had a lower frequency of MSI-H (15% vs. 42%; $p = .015$) [25].

Conclusion

In conclusion, considering that *KRAS* mutations confer resistance to EGFR inhibitors, patients who have CRC with *KRAS* mutation could receive more tailored management by defining MSI status. MSI-high patients have enhanced responsiveness to anti-PD-1 therapies. Thus, the question arises as to whether it is worth investigating this association in the routine clinical setting or not. Further studies with a larger number of patients are needed to assess the impact of MSI status on Egyptian CRC care.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by the Kasr Al Ainy Clinical Oncology Department Institutional Review Board (IRB)-3-2018. Informed consent was obtained from all individual participants included in the study. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments (GCP guidelines) or comparable ethical standards.

Consent for publication
The consent to publish had been taken from each participant in this work.

Competing interests
The authors declare that they have no competing interests.

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