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Colorectal Cancer in Morocco: Is There a Correlation between the Epidemiological and Anatomopathological Profile and the RAS Status?

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Abstract

Introduction: Colorectal cancer is the third most common cancer in Morocco. The profile of patients according to RAS status has been the subject of few studies in Morocco. The objective of our study is to evaluate the epidemiological, anatomopathological and molecular parameters of a Moroccan population according to RAS status and to search for a possible correlation between the latter and the RAS mutation. **Material and Methods:** This is a cross-sectional study conducted at the Mohamed VI Center for Cancer Treatment in Casablanca during the period from May 2015 to February 2021. Included were 282 patients with histologically confirmed colorectal cancer whose RAS status was known and significant. The main criteria studied were age, gender, personal and family history of colorectal cancer or other types of cancers, anatomo-pathological characteristics (tumor site, histological type, tumor size, lymph node invasion, laterality, differentiation grade, stage, BRAF, MSI…), therapeutic modalities and evolutionary data. **Results:** 47.2% of patients were RAS Wild Type and 52.8% of whom were RAS mutated with a predominance of KRAS mutation (92.6%). The analysis of the association between the RAS mutation and the epidemiological and anatomopathological characteristics of the patients did not reveal any statistically significant correlation, except for two parameters: age ($p = 0.035$) and degree of differentiation ($p < 0.003$). Thus, mutated RAS status was associated with advanced age and good differentiation of colorectal adenocarcinomas. The evolution of the patients under treatment as well as their prognosis did not show any statisti-

cally significant difference between the two wild-type and mutated RAS groups. **Conclusion:** The latter was not correlated with the anatomopathological and epidemiological characteristics of the patients except for the advanced age and the well-differentiated type of colorectal adenocarcinoma.

Subject Areas

Gastroenterology & Hepatology, Oncology

Keywords

Colorectal Cancer, RAS Status, Epidemiological and Anatomopathological Profile

1. Introduction

Colorectal cancer is a public health problem. It affected nearly 1.9 million new cases and caused 930,000 deaths in 2020; this ranks it as the third most common cancer and the second cause of death worldwide [1]. It accounts for 7.4% of the cancer cases diagnosed in the Middle East and North Africa region [2]. This frequency rate remains lower than that observed in Western countries, particularly in Europe and North America [3]. In Morocco, colorectal cancer is the third most frequent cancer after breast and lung cancer. It reaches a rate of 6.9% of cancers in women and 8.9% in men according to the latest cancer registry of Greater Casablanca. Its incidence is increasing and it is currently the first digestive cancer [4].

Colorectal cancer is one of the examples that illustrate the multi-step process of carcinogenesis caused by genetic and epigenetic mutations, which will be responsible for the inactivation of anti-oncogenes and the activation of proto-oncogenes. Among the latter, we find the RAS family. RAS proteins are GTPases located on the inner side of the cytoplasmic membrane and anchored in the membrane phospholipid layer by their C-terminus. There are 3 RAS genes encoding four isoform proteins: HRAS, NRAS and the two splice variants of the KRAS gene, KRAS4a and KRAS 4b containing exons 4a and 4b respectively. The activation of RAS proteins is triggered through membrane receptors, the most important of which is EGFR. Ligand binding to EGFR will lead to a RAS/RAF/MAPK phosphorylation cascade and thus to activation of transcription of genes involved in proliferation, differentiation, migration, cell invasion and angiogenesis [5]. The presence of a mutation in the RAS genes leads to a constitutive activation, independent of ligand binding, of the RAS downstream signaling pathways and thus to tumor progression. This was confirmed in 1987 by the team of B. Vogelstein who reported a high rate of KRAS mutations reaching 40% in a series of 27 cases of colorectal cancer at an early phase of colorectal carcinogenesis, and highlighted for the first time the involvement of RAS genes in colorectal cancer

[6].

Testing for RAS mutation status is currently an integral part of the management of metastatic colorectal cancer, as RAS mutation leads to resistance to targeted therapies. Some studies have shown a correlation between RAS mutation and epidemiological and anatomopathological characteristics of patients. These studies have been carried out in the majority of cases in the West and therefore rare studies exist in the East and two studies have been carried out in Morocco. The first one is at the Mohammed VI cancer treatment center on metastatic colorectal cancers [7] and the second one is at the university hospital of Fez. [8] The objective of our work is to study the epidemiological and anatomopathological characteristics of a Moroccan population with localized or metastatic colorectal cancer, and to raise the presence or not of a correlation between these parameters and the RAS mutation.

2. Material and Methods

2.1. Research Method

A cross-sectional study was carried out at the Mohamed 6 Center for cancer treatment in Casablanca, one of the largest public oncology centers in Morocco.

All colorectal cancer cases verified microscopically for which the RAS status could be determined and managed at the center during the study period were included.

Data collection was performed from the patients' files and pathology reports, studying the following parameters: Age, gender, personal and family history of colorectal cancer or other types of cancers, clinical and anatomopathological characteristics (tumor site, histological type, tumor size, lymph node invasion, laterality, differentiation grade according to the old WHO 2010 classification, stage, BRAF, MSI…), therapeutic modalities and evolutionary data (remission, stability, recurrence, progression, death). These data were indexed according to RAS status. Molecular testing of RAS status was performed in the anatomopathology laboratory of the Mohamed VI center. BRAF, PI3K and MSI mutation analysis was not performed in all patients and was done at the request of the treating physician.

2.2. Statistical Analysis

First, a description of demographic characteristics of the patients was made by calculating percentages for qualitative variables such as gender, personal and family history of colorectal cancer or other types of cancers, and anatomopathological characteristics like tumor site, histology type, lymph node invasion, laterality, and grade or by estimating means with a standard deviation for quantitative variables such as age, tumor size.

Afterward, the comparison of the percentages was done to verify the association between the RAS status and the characteristics of the patients using the Chi^2 test or Fisher's exact test when the theoretical number of patients is lower than 5.

Comparison of means to verify the association of quantitative variables such as age and tumor size with RAS status was performed using Student's t test or ANOVA.

Statistical analysis was performed by R software. Tests were statistically significant when $p < 0.05$.

3. Results

3.1. Epidemiological Characteristics

282 patients were included in our study. The mean age was 56.7 years with extremes of 19 and 92 years (SD = 12.7). 148 patients were female and 134 male with respective proportions of 52.5% and 47.5%.

A personal history of colorectal cancer was noted in 15 patients, *i.e.*, 7.2% of cases. A history of other cancers was reported by 4 patients (1.4% of cases).

A family history of colorectal cancer was noted in 33 patients (11.7%). A family history of other cancers of the Lynch syndrome was observed in 21 patients (7.4%). Three patients had diseases predisposing to colorectal cancer including 1 case of IBD (0.3%) and 2 cases of FAP (0.7%). The epidemiological characteristics of the patients are summarized in Table 1.

3.2. Anatomopathological and Molecular Characteristics

The anatomopathological study was performed on a tumor biopsy in 163 patients and on an operative specimen in 119 patients, *i.e.*, with respective rates of 57.8% and 42.2% of cases.

Concerning the tumor site, left colon cancers were the most frequent (n = 140, 49.6%) followed by rectal cancers (n = 93, 33%) and right colon cancers (n = 49, 17.4%). The mean tumor size in our study was 5.6 cm (SD = 2.2).

The most frequent histological subtypes were Lieberkuhnian adenocarcinoma (n = 255) followed by mucinous adenocarcinoma (n = 24) with respective rates of 90.4 and 8.5%. The presence of vascular emboli was noted in 54 patients, *i.e.* 19.2% of cases. Perineural emboli were found in 36 patients or 12.8% of cases.

The tumor grade was distributed according to the WHO 2010 classification as follows: 57.4% of the colorectal tumors were moderately differentiated, 29.8% were well differentiated and 12.8% were poorly differentiated or undifferentiated. Positive lymph nodes were found in 22.3% of the patients studied.

Stage IV colorectal cancer (n = 189) accounted for 67%, followed by stage III (n = 15), II (n = 8) and I (n = 1) with rates of 5.3, 2.8 and 0.3% respectively. In stage IV patients, metastases involved a single site in 47.08% of cases, 2 sites in 37.6%, 3 sites in 8.99%, 4 sites in 2.1% and more than 4 sites in 1.05% of cases.

The liver was the most frequent metastatic site and involved 117 patients, *i.e.* a rate of 61.9%, followed by the lung (n = 79), the peritoneum (n = 59) and the bone (n = 8), *i.e.* rates of 41.7%, 31.2% and 4.2% respectively.

Of the 282 patients studied, 149 patients had a mutated RAS status, *i.e.*, 52.8% of the cases, while 133 patients had a wild-type RAS status, *i.e.*, 47.2%.

BRAF status was analyzed in 156 patients, with a mutation in 5 patients (3.2%).

The search for microsatellite instability was performed in 57 patients, revealing an MSI status in 9 patients (15.8%).

PI3K status was performed in 155 patients, showing a mutation in 30 patients (19.3%). The main anatomopathological and molecular characteristics are summarized in Table 1.

Table 1. Epidemiological anatomopathological and molecular characteristics of patients.

	Number	%
Mean age (mean SD)	56.7	SD = 12.7
Gender		
Female	148	52.5
Male	134	47.5
Personal history		
CRC	15	5.3
Other cancers	4	1.4
Family history		
CRC	33	11.7
Lynch Syndrome	21	7.4
Predisposing diseases		
FAP	2	0.7
IBD	1	0.3
Tumor site		
Right colon	49	17.4
Left colon	140	49.6
Rectum	93	33.0
Histological subtype		
Lieberkuhnian	255	90.4
Mucinous	24	8.5
Others	3	1.0
Tumor grade		
Well	84	29.8
Moderate	162	57.4
Poor	36	12.8
Mean tumor size (cm) (mean SD)	5.6	SD = 2.2

Continued

Vascular invasion		
Yes	54	19.2
No	46	16.3
Not specified	182	64.5
Perineural invasion		
Yes	36	12.8
No	42	14.9
Not specified	204	72.3
Disease stages		
I	1	0.3
II	8	2.8
III	15	5.3
IV	189	67.0
Not specified	69	24.5
Lymph node invasion		
N0	31	11.0
N+	61	21.6
Not specified	190	67.4
Distant metastases		
Liver	117	61.9
Lung	79	41.7
Peritoneum	59	31.2
Bone	8	4.2
Others	17	6.0
RAS		
Wild Type	133	47.2
Mutated	149	52.8
BRAF		
Wild Type	151	96.8
Mutated	5	3.2
MSI		
MMR	48	84.2
MSI	9	15.8
PI3K		
Wild Type	125	80.6
Mutated	30	19.4

3.3. Frequency of RAS Mutations and Type of Molecular Substitutions

The RAS mutation concerned the KRAS gene in 138 patients *i.e.* 92.6% of cases and the NRAS gene in 11 patients or 7.4% of cases.

In the NRAS mutated group, the mutation was present on exon 3 in 9 cases (81.8%) and exon 2 in 2 cases (18.2%). In the KRAS mutated group, the mutation was present in exon 2 in 130 patients (94.2%), exon 3 in 2 patients (1.5%) and exon 4 in 6 patients (4.3%).

The most predominant substitutions in the KRAS group were: c.35G > A (p.G12D), c.35G > T (p.G12V) and c.38G > A (p.G13D) with percentages of 31.5%, 26.8% and 19.5% respectively. Concerning the NRAS group, 5 types of substitutions were found. The c.181C > A mutation (p.Q61K) was the most frequent substitution (2.7%), followed by the c.181A > G mutation (p.Q61R) (1.3%). Table 2 summarizes the different substitutions observed in the 2 KRAS and NRAS groups.

Table 2. Frequency and distribution of NRAS and KRAS mutations.

Gene alterations	Exon	Nucleotide substitution	Codon Substitution	Number (n = 149)	Percentage (%)
KRAS	2	c.35G > A	G12D	47	31.5
	2	c.35G > T	G12V	40	26.8
	2	c.38G > A	G13D	20	19.5
	2	c.34G > T	G12C	10	6.7
	2	c.35G > C	G12 A	9	6.04
	2	c.34G > A	G12S	3	2.01
	2	c.37G > T	G13C	1	0.7
	3	c.175G > A	p.A59T	1	0.7
	3	c.182A > T	p.Q61L	1	0.7
	4	c.436G > A	p.A146T	3	2.01
	4	c.437C > T	p.A146V	1	0.7
	4	c.351A > T	p.117A	2	1.3
NRAS	3	c.181C > A	p.Q61K	4	2.7
	3	c.181A > G	p.Q61R	2	1.3
	3	c.183A > C	p.Q61H	1	0.7
	2	c.35G > A	p.G12D	2	1.3
	3	c.182A > G	Glu61Arg	2	1.3

3.4. Association between RAS Status and Epidemiological and Anatomopathological Features

The analysis of the association between RAS mutation and the epidemiological and anatomopathological characteristics of the patients did not reveal statistically significant correlations, except for two parameters: age ($p = 0.035$) and tumor grade ($p < 0.003$).

Indeed, mutated RAS status was associated with advanced age (mean age 58.3 vs 55.1) and well differentiation of colorectal adenocarcinomas.

Statistical analysis of the association between RAS mutation and epidemiologic and pathologic features was summarized in Table 3.

3.5. Therapeutic Modalities

134 patients underwent surgery of which 62 patients were WT RAS and 72 patients were mutated RAS. In 22 patients, the resection of the primary cancer was curative (stage I: 1, stage II: 7, stage III: 14).

For palliative purposes, 25 patients underwent colostomy and 56 patients had resection of the primary cancer. These patients were all metastatic.

Resection of the primary cancer and a single metastatic site was performed in 11 patients, 9 of whom had liver metastasis and 2 had ovarian metastasis.

Regarding systemic treatment, chemotherapy was received by 182 patients of whom 98 patients had mutated RAS status and 84 patients had WT RAS status.

The latter was administered for adjuvant purpose in 24 patients, 6 of whom had stage IV colon cancer with a single resected liver metastasis, 4 had high-risk stage II cancer and 14 had stage III cancer.

6 patients received neoadjuvant chemotherapy, including one patient with stage III colon cancer and five patients with colorectal cancer with a single metastatic site.

For palliative purposes, 152 patients received chemotherapy for multi-metastatic colorectal cancer.

32 patients received radiotherapy, 17 of whom were in the mutated RAS group and 15 in the WT RAS group. Their distribution was as follows: 26 patients received radiotherapy combined with neoadjuvant chemotherapy for localized rectal tumors, and 6 patients received palliative radiotherapy.

Regarding targeted therapies, 77 patients received an anti-VEGF antibody of the Bevacizumab type, of which 54 had mutated RAS and 23 had WT RAS. The anti-EGFR antibodies were administered exclusively in patients with WT RAS of which 6 patients had Cetuximab and 26 patients had Panitumumab.

3.6. Therapeutic Evaluation and Prognosis

The therapeutic evaluation of patients was performed after 12 and 24 weeks of the first line of chemotherapy with FOLFOX or FOLFIRI in metastatic patients according to RECIST criteria.

Excluding patients who were lost to follow-up, *i.e.*, 76 patients (27% of the cases), the evolutionary data of 206 patients are available.

In the mutated RAS group, the evolution of the patients was as follows: 7 patients presented a recurrence (4.7%), 31 patients a stability (19.1%), 4 patients a complete remission (2.7%) and 43 patients a progression (28.9%).

Compared to the wild-type RAS group, 2 patients showed complete remission (1.5%), 23 patients showed stability (17.3%) and 45 patients showed progression (33.8%).

14 patients treated for localized cancer had metastatic recurrence (10.5%).

Death was noted in 17 patients in the wild-type RAS group and 20 patients in the mutated RAS group, *i.e.*, with respective rates of 12.8% and 13.4%.

However, no statistically significant correlation was noted between RAS status and patient outcome.

The association between patient outcome and RAS status is summarized in Table 4.

Table 3. Association between RAS mutation and epidemiological and pathological characteristics of patients.

Characteristics	Wild-type RAS	Mutated RAS	P
Mean age	55.1	58.3	0.035
≤55	57 (42.9%)	58 (38.9%)	
>55	76 (57.1%)	91 (61.1%)	
Gender			
Female	64 (48.1%)	84 (56.4%)	0.166
Male	69 (51.9%)	65 (43.6%)	
Personal history			
CRC	6 (4.5%)	9 (6%)	0.567
Other cancers	1 (0.8%)	3 (2%)	0.591
Family history			
CRC	19 (14.3%)	14 (9.4%)	0.188
Other cancers	7 (5.3%)	14 (9.4%)	0.629
Tumor site			
Right colon	28 (21.1%)	21 (14.1%)	
Left colon	60 (45.1%)	80 (53.7%)	0.147
Rectum	45 (33.8)	48 (32.2%)	
Histological subtype			
Lieberkuhnian	123 (92.5%)	132 (88.6%)	
Mucinous	7 (5.3%)	17 (11.4%)	0.137
Others	3 (2.2%)	0	
Tumor grade			
Well	29 (21.8%)	55 (36.9%)	
Moderate	79 (59.4%)	83 (55.7%)	0.003
Poor	25 (18.8%)	11 (7.4%)	

Continued

Mean tumor size	5.34	5.93	0.423
Vascular invasion			
Yes	26 (19.5%)	28 (18.8%)	
No	23 (17.3%)	23 (15.4%)	0.854
Not specified	84 (63.2%)	98 (65.8%)	
Perineural invasion			
Yes	19 (14.3%)	18 (12%)	
No	18 (13.5%)	24 (16.1%)	0.382
Not specified	96 (72.2%)	107 (71.8%)	
Diseases stages			
I	1 (0.8%)	0	
II	4 (3%)	4 (2.7%)	
III	8 (6%)	7 (4.7%)	0.689
IV	87 (65.4%)	102 (68.4%)	
Not specified	33 (24.8%)	36 (24.2%)	
Lymph nodes invasion			
N0	15 (11.3%)	16 (10.7%)	
N+	27 (20.3%)	34 (22.8%)	0.312
Not specified	91 (68.4%)	99 (66.4%)	
Distant metastases			
Liver	57 (65.5%)	60 (58.8%)	0.421
Lung	32 (36.8%)	47 (46.1%)	0.151
Peritoneum	26 (29.9%)	33 (32.3%)	0.644
Bone	4 (4.6%)	8 (7.8%)	0.396
MSI			
MMR	17 (12.8%)	31 (20.8%)	
MSI	5 (3.6%)	4 (2.7%)	0.255
Not specified	111	114	
BRAF			
WT	52 (39.1%)	99 (66.4%)	
Mutated	5 (3.8%)	0	0.037
Not specified	76 (57.1%)	50 (33.6%)	
PI3K			
WT	48 (36.1%)	77 (51.7%)	
Mutated	8 (6%)	22 (14.8%)	0.169
Not specified	77(57.9%)	50 (33.5%)	
Total	**133**	**149**	

Table 4. Association between patient outcome and RAS status.

	RAS Wild Type (%)	Mutated RAS (%)	P
Remission	2 (1.5)	4 (2.7)	0.504
Recurrence	14 (10.5)	7 (4.7)	0.074
Stability	23 (17.3)	31 (19.1)	0.797
Progression	45 (33.8)	43 (28.9)	0.854
Death	17 (12.8)	20 (13.4)	0.950

4. Discussion

This is the first study of RAS status done in a representative population of the Moroccan population given that the Casablanca region contains 10% of the Moroccan population. This study follows the first study presented at ESMO 2017 with a smaller sample size including only patients with metastatic colorectal cancer [7] and the study of the population of the Fez region [8]. The prevalence of the RAS mutation found in our series is 52.8%. This mutation rate is superimposed on that found by several studies, carried out in different countries, which have searched for the RAS mutation in exons 2, 3 and 4 of the KRAS and NRAS genes.

In the Western region, we first cite the study by Serebriiskii *et al.* in the United States, where the prevalence of RAS mutation was 56.3%, among 13,336 colorectal cancer cases, all stages combined [9]. In Australia, the RAS mutation was estimated to be 55.2% among 159 metastatic colorectal cancers managed between 2013 and 2018, according to Kuchel *et al.* [10]. According to a Canadian study done by Loree *et al.* that included 242 patients with metastatic colorectal cancer, 57% of cases had mutated RAS [11].

In a larger analysis by Peeters *et al.* which pooled data from 3 phase III randomized controlled trials, the prevalence of RAS mutation was also consistent with the above studies and reached 55.9% of cases; the patients recruited were from Europe and America and all had metastatic colorectal cancer [12].

However, if we compare the patients of our study with other Arab populations, we find that the prevalence of the RAS mutation is higher than that observed in Egypt, Saudi Arabia or Jordan where it does not exceed the respective rates of 31.6%, 42.2% and 44% [13] [14] [15]. Indeed, a large observational study by Kafatos *et al.* confirms this finding, noting through 4431 patients with colorectal tumors from 12 countries, half of which are represented by Arab countries (Algeria, Bahrain, Egypt, Kuwait, United Arab Emirates and Saudi Arabia) in addition to the regions of South America, Central and Eastern Europe, a prevalence of RAS mutation that varies between 33.7% and 34.6% in Arab countries versus 53.6% and 54.1% in European centers [16].

In the same way, a direct comparison between an American population and an Arab population from the Gulf countries was carried out by Al Shamsi *et al.* but did not show a great difference in the RAS mutation with respective rates of

48.4% and 44.4% [17].

By analyzing these different studies, we can see that some modifiable factors can be incriminated in the increase of the prevalence of the RAS mutation in the western region compared to the eastern one. And among these factors, we note the specificities of the diet of each region. Indeed, Arab countries are characterized by lower consumption of alcohol and red meat, and an abstention from pork consumption among Muslims, unlike the West.

In this sense, Sattery *et al.* demonstrated in their study analyzing the involvement of dietary habits in the variation of the RAS profile, a correlation between high alcohol consumption and a mutated RAS [18].

The same result was also demonstrated by Jayasekara H. *et al.* in 2016 [19]. The involvement of alcohol in the occurrence of RAS mutation seems significant in the case of a high level of consumption; in the case of moderate alcohol consumption, the risk of developing colorectal tumors is still present but seems to be due to mechanisms other than those responsible for KRAS mutations [20].

Carr *et al.* have shown a correlation between high red meat consumption and the RAS mutation [21]. The mechanism that seems to induce the latter is the induction of the endogenous production of N-nitroso compounds and their precursors, which will be involved in the occurrence of the KRAS mutation [20].

However, the high prevalence of the KRAS mutation in our study, which is similar to the rate found in Western countries and appears to be slightly higher than in other Arab populations, could be explained by the gradual abandonment of the Mediterranean diet in favor of a diet that is increasingly similar to Western countries.

The role of dietary habits in the increased prevalence of the RAS mutation remains to be confirmed by further comparative prospective studies.

In relation to the spectrum of RAS mutation, the prevalence of mutations in the KRAS and NRAS genes was 92.6% and 7.4% of all cases, respectively. This rate is consistent with data from several studies, reporting a higher frequency of KRAS mutations (33% - 54%), compared to NRAS mutations (2% - 7%) in colorectal cancer [17] [22] [23] [24].

In the KRAS group, the majority of mutations detected were present in exon 2 (93.25%), whereas in the NRAS group, mutations identified in exon 3 were predominant (6%), a result that is superimposed on that found in the study by Peeters *et al.* [12].

The implication of age in the occurrence of colorectal cancer is already established. However, its correlation with the presence or absence of an RAS mutation has not yet been confirmed.

Our study was able to identify this finding by showing a slightly higher average age in patients with a mutated RAS (58.3 vs 55.1) with a significant p at 0.035. However, several studies did not raise this correlation, namely the analysis of Peeters *et al.* which did not show a statistically significant difference between the two groups of wild type RAS and mutated RAS despite a predominance of

the age group between 50 and 69 years of age of the RAS mutation cases, with a rate of about 64.2% [12].

Kafatos *et al.* also noted no difference in the prevalence of RAS by age. The latter was 42.9 and 40.9% in the age groups 18 - 49 and 50 - 69 years respectively [16]. Other European studies did not find the involvement of age in RAS mutation [25] [26]. The same finding was demonstrated in a study conducted in the Gulf countries [17].

Nevertheless, an Egyptian study found a concordant result with our study by demonstrating a mutated KRAS in older patients with a significant p of 0.003 [13]. However, the sample size in this study was very small and therefore the hypothesis of an association between age and RAS profile needs to be evaluated in future studies with larger numbers to be confirmed.

The study of the distribution of patients according to RAS status showed a predominance of the female sex in the case of RAS mutation (56.4% vs 43.6%). However, the association between RAS mutation and the sex of the patients was not demonstrated and was not statistically significant.

This result is supported by other studies, namely that of Peeters *et al.* who found a slightly higher prevalence of the RAS mutation in women (58.7% vs. 54.2%) without being statistically significant [12], and that of Kafatos *et al.* who, through their meta-analysis of 12 observational studies in 12 different countries, reported an absence of association between the sex of the patients and the RAS mutation, with a female proportion of 43.3% and a male proportion of 43.8%, *i.e.* a sex ratio (M/F) balanced at 1.01 [16].

No difference was also noted in the Gulf countries between the two sexes regarding RAS status [17].

Concerning the family history of patients, a history of familial colorectal cancer was found in our study in 9.4% of cases with mutated RAS. This rate increases to 14.3% in patients with wild-type RAS, without a statistically significant correlation between the history of colorectal cancer and RAS status.

Similar results were reported by a prospective study in the Netherlands by Brink *et al.* on 737 cases of colorectal cancer, where 11% of patients with wild-type RAS had a family history of colorectal cancer compared with 9% of patients with mutated KRAS. However, this difference was not statistically significant ($p = 0.21$) [27].

Slattery *et al.* did not find any influence of family history of colorectal cancer on the RAS status of the patients, the distribution of the latter was similar in the mutated and wild type RAS groups with respective rates of 15.1% and 15.6% [28]. The same finding was noted by Al Shamsi *et al.* who did not raise a significant difference according to KRAS status (KRAS-mutated = 9.1% vs KRAS-WT = 6.1%; $p = 0.703$) [17].

This suggests that the RAS mutation appears to be more incriminated in sporadic forms of colorectal cancer where gene alterations are due to exposure to modifiable risk factors, namely dietary habits, lifestyle and environmental fac-

tors. In mutated RAS patients with a family history of CRC, colorectal carcinogenesis could be explained by the association of a hereditary component with modifiable risk factors.

Our study also evaluated the correlation between anatomopathological parameters and RAS status; these parameters include laterality, histological subtype, tumor grade, tumor stage, type and site of metastasis.

The laterality of colorectal cancer has been the subject of several discussions, distinguishing certain differences in terms of epidemiological, anatomopathological, molecular and prognostic criteria between right and left colorectal cancer locations. Indeed, cancers of the right colon are associated with female gender, older age, and poorly differentiated mucinous tumors with a high rate of BRAF mutations and a poorer prognosis; whereas cancers of the left colon are associated with male gender and EGFR amplification, hence their sensitivity to anti-EGFR drugs and their more favorable prognosis.

The variation of the RAS profile according to the laterality of the colorectal cancer was not raised by our study and a predominance of left colorectal cancer was found in both wild type (45.1%) and mutated (53.7%) RAS groups. Similar to our work, Kafatos *et al.* did not show a significant difference between the RAS status of the patients and the laterality of colon cancer [16].

The study of the correlation between the RAS status and the site of the colon tumor in Arab populations, notably Egypt and Saudi Arabia, also did not show a significant correlation [13] [14].

Two studies performed on larger series of colorectal cancers showed different results from the previous studies; the first one was performed on 194 laboratories of Pathological Anatomy across Europe and found a higher prevalence of RAS mutations in right colon cancers (54.6%) compared to left colon cancers (46.4%) [29]. The second series was a meta-analysis of 42 studies including 15,981 metastatic colorectal cancers tested for RAS, and found a significant association between the presence of the RAS mutation and right colon site ($p = 0.017$) [30].

The contrast observed between the latter two studies and our series may be due to the high proportion of patients with left colon cancer recruited in the latter, in both wild-type and mutated RAS groups.

Regarding histological subtype, Lieberkuhnian adenocarcinoma was the most predominant histological subtype in our study, with a rate reaching 92.5% and 88.6% in the wild-type and mutated RAS groups respectively. The mucinous subtype came in 2nd position with a higher percentage in the mutated RAS group (11.4% vs 5.3%).

Lieberkuhnian adenocarcinoma was also the most representative subtype by other studies including Rimbert *et al.* (90.7%) [31] and Guo *et al.* (88.6%) [32] in patients with mutated RAS. The mucinous subtype occupied the 2nd position (8.7% and 5.2% respectively) [31] [32]. In Arab populations from Saudi Arabia, United Arab Emirates, Kuwait, Qatar, Bahrain and Oman through the study of

Al Shamsi *et al.* the distribution of Lieberkuhnian adenocarcinomas was almost similar in the 2 groups of wild type and mutated RAS with respective rates of 50.6% and 49.4% while the rate of mucinous adenocarcinomas was more important in the wild type group. However, no correlation between histological type and RAS status was noted [17].

The analysis of the association between RAS mutation and histological subtype has been the subject of conflicting data. As a result, some authors find no statistically significant correlation as was noted in our series as well as those of Guo *et al.* [32] and Al Shamsi *et al.* [17]. Other authors such as Rimbert *et al.* concluded that mutated RAS colorectal cancers were significantly associated with the Lieberkuhnian subtype [31].

Although not all patients were investigated, our study did not find a statistical correlation between the presence of vascular or perineural invasion and the RAS mutation; this finding is consistent with the results found by Rimbert *et al.* [31] and Guo *et al.* [32].

In the same way, the mean tumor size was almost similar in the 2 groups RAS wild type and mutated and no significant association with RAS mutation was noted; this has been objectified by several other studies in both the West and the Middle East [13] [33] [34] [35].

The tumor grade of colorectal cancers has long been known to be a poor prognostic factor; this parameter was explored by our study which found that the RAS mutation was significantly associated with low grade colorectal adenocarcinomas ($p = 0.03$). This result is in agreement with that found by Rimbert *et al.* (93.4%, $p < 0.0001$) [30]. Other studies have demonstrated this association, particularly in the case of KRAS mutation [36] [37] [38].

These superimposable results suggest that the RAS mutation is involved in the genesis of low-grade tumors, which suggests that the occurrence of high-grade colorectal cancer could be due to other mutations with a more aggressive tendency, such as the BRAF mutation, which has already been noted by some studies to be correlated with poor differentiation [39] [40] [41].

The involvement of the RAS mutation in the tumor stage of colorectal cancer has not been proven in our population; indeed, the association between metastatic tumors and the RAS mutation is not statistically significant and a predominance of the latter was noted in the 2 RAS wild-type and mutated groups. This is in accordance with several studies including the meta-analysis of Kafatos *et al.* [16], the study of Guo *et al.* [32] and Al Shamsi *et al.* [17].

Therefore, the RAS mutation seems to occur at an early stage of the colorectal cancer process and may be present in localized cancers. Nevertheless, the search for the RAS mutation is currently of interest in clinical practice only in stage IV colorectal cancers, since it makes it possible to orientate a treatment by a targeted therapy, which could partly explain the predominance of stage IV colorectal cancers in our study. This predominance can also be explained by the low socio-economic and cultural level of the population treated at our facility as well as

the absence of a generalized colorectal cancer screening policy in Morocco, resulting in a delay in diagnosis.

Similarly to the tumor stage, the RAS mutation does not seem to influence the type of metastasis of colorectal cancers.

Indeed, the liver occupied the first position of metastatic sites in our study with respective rates of 65.5% and 58.8% in wild-type and mutated RAS patients. The lung and the peritoneum represented the 2nd and 3rd most frequent metastatic site respectively. However, RAS mutation was not significantly associated with the type of metastasis, which was consistent with the analysis of Kafatos *et al.* and Peeters *et al.* who found similar results [12] [16].

In contrast to our study, Bader *et al.* found a predominance of isolated lung metastases in the mutated KRAS group compared to the wild-type KRAS group in a Saudi population with respective rates of 32% and 3% and concluded that there was a statistically significant correlation between KRAS mutation and the occurrence of isolated lung metastases with a $p < 0.005$ [14]. Similarly, Tie *et al.* found that lung and brain metastases were significantly associated with a mutated RAS profile [42].

These last two studies suggest that the presence of a RAS mutation may lead to migration of tumor cells, without transiting through an essential site such as the liver, to other organs, notably the lung. However, some liver lesions may go unnoticed and not be visualized on the initial baseline radiological workup; this may explain their absence in these RAS mutated patients. These data should be analyzed with caution taking into account the tumor site on the colon. Indeed, the double venous return to the portal vein and inferior vena cava in middle and lower rectal cancer could explain the more frequent and earlier occurrence of secondary pulmonary localizations.

The impact of the RAS mutation on the prognosis of patients has been the subject of controversy.

Some studies have not shown an association between RAS mutation and a decrease in overall survival and progression-free survival. These include studies by Ince W. L. *et al.* and Westra *et al.* published in the early 2000s [43] [44] [45]. More recent studies, such as the one performed by Ottaiano *et al.* on a sample of 446 patients with metastatic colorectal cancer, have shown that overall survival was lower in the mutated RAS group, hence its association with a poor prognosis [46].

Our study has some limitations: we did not investigate the correlation between other mutations, namely BRAF, MSI and PI3K, and epidemiological and anatomopathological factors, as these mutations were not systematically searched in all patients.

The sample of localized tumors is poorly represented since the RAS status is requested mainly for the research of anti-EGFR sensitivity.

Our study assessed the RAS status in the Mohammed VI cancer treatment centre, which caters to low and middle income cancer patients. Our results can-

not be generalised to the Moroccan population as data on high income patients treated in the liberal sector are missing. However, our centre draws cancer patients from the whole of southern Morocco. It is the second centre after the National Institute of Oncology in Rabat in terms of the volume of patients treated, unlike the other oncology centres which drain patients from the towns where they are located.

5. Conclusion

Our work has shown the correlation between RAS status and age and the degree of differentiation with a female predominance without being significant among all the factors studied. The percentage of RAS mutation is higher than in the Arab and Muslim populations but remains comparable to the Western population reflecting the epidemiological transition in our context. Our results need to be confirmed by larger studies involving more high-income populations treated in the liberal sector in order to generalise.

Conflicts of Interest

The authors declare no conflicts of interest.

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