

K-ras in Colorectal Cancer Tumors From Saudi Patients: Frequency, Clinico-pathological Association and Clinical Outcome

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Abstract: *Background:* K-ras oncogene mutations are confirmed factor for lack of clinical benefit from antibodies targeting EGFR in patients with colorectal cancer (CRC). Mutations are reported in 40% of CRC tumors in western patients. There is scarcity of data on population from Asia and the Middle East.

Aims: This study investigates the frequency and impact of k-ras mutation and the association between clinico-pathological features and K-ras status in Saudi patients with CRC.

Patients and Methods: Retrospective review of K-ras status, clinico-pathological characteristics and clinical outcome in 46 patients with CRC.

Results: K-ras mutations were identified in 15/46 (32%) tumors, 87% at codon 12 and 13% at codon 13. Gender, site of tumor, stage at diagnosis, differentiation and lymphatic and vascular invasion were tested as potential risk predictors for K-ras status. Only gender was found to be a potential risk factor. Female gender compared to male posed higher significant chance of wild type status (RR=1.54, 65% CI: 1.07-2.2; P=0.034). K-ras status (mutant vs. wild) did not statistically significantly impact on clinical outcome as measured by development of relapsed disease (80% vs. 81%), median relapse free survival (17 vs. 11 months, P=0.256) and overall survival (not reached in both groups, P=0.59).

Conclusion: This relatively small retrospective series shows that rate of K-ras mutation in Saudi patients with CRC is lower than reported in western Caucasian population but close to rates reported in neighboring Asian population. Mutations are less frequent in females. In these patients, K-ras mutation status did not significantly impact clinical outcome.

Keywords: Asia, Colorectal cancer, K-ras, Mutant, Wild type, Saudi.

INTRODUCTION

Colorectal cancer (CRC) is the third and fourth most common cancer in women and men worldwide, respectively and the fourth commonest cause of cancer death [1]. The outcome of treatment of patients with a metastatic CRC has improved in the past decade. This improvement was mainly due to the introduction of both active new chemotherapeutic agents [2] and novel targeted drugs [3].

Cetuximab is a chimeric IgG1 monoclonal antibody with high selectivity and affinity for the epidermal growth factor receptor (EGFR), which is over-expressed by 25–80% of colorectal cancer tumors [4, 5]. Clinically, cetuximab demonstrated the ability to evade chemotherapy resistance [6] and improved response rate (RR) and progression free

survival (PFS) when combined with FOLFIRI in the first line setting [7].

Until recently, the most consistent predictor for response and survival to cetuximab is the development of skin rash [6]. K-ras (Kirsten-ras) oncogene mutation occurs in 58% of colonic adenomas larger than 1 cm and in 47% of CRC [8].

The K-ras gene encodes the K-ras protein that regulates 2 signaling pathways: PI3K/AKT and RAF/MEK/ERK. These pathways control cell proliferation, differentiation and apoptosis. Most frequent mutations are in codons 12 and 13 from exon 2. Over the last few years, increasing number of reports confirmed the correlation between K-ras gene status (mutant vs. wild type) and clinical benefit from cetuximab [9]. Now cetuximab based therapy is indicated only in patients with tumors expressing wild type K-ras genotype.

Collectively four randomized studies included a total of 2,292 mostly western patients with known k-ras status. 1,389 (60%) and 903 (40%) patients had wild and mutated K-ras tumors respectively [9]. Most of data on frequency and impact of k-ras status is reported on Western population and

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there scarcity of data on population from Asia and the Middle East.

The aim of this retrospective study is to investigate the frequency and impact of k-ras mutation at codons 12 and 13 and the association between clinico-pathological features and K-ras status exclusively in Saudi patients with CRC.

MATERIALS AND METHODOLOGY

46 consecutive CRC samples from 46 Saudi patients were examined for K-ras status purely for clinical indication and as requested by the treating oncologist. Patients were diagnosed with CRC with in the period of November 2003 and January 2011. The first sample in this series was tested for K-ras status in October 2009 and last in February 2011. All data were retrospectively collected by the investigators over a period of one month from 15 March 2011 until 15 April 2011.

K-ras mutation analysis was performed by Biomnis laboratory. Formalin fixed paraffin embedded material from colorectal carcinomas were used for DNA extraction. Array kit was used for detection of mutations within codons 12 and 13 of K-ras. The mutations tested are p.G12S, p.G12R, p.G-

12C, p.G12D, p.G12A, p.G12V ,p.G13D, p.G13D, p.G13R.

Tumor and patients characteristics were retrospectively extracted from paper and electronic records by 2 medical oncologists.

Clinico-pathological features selected for association testing included gender, site of tumor, AJCC stage at diagnosis, tumor differentiation, vascular invasion and lymphatic invasion. Tumors of the ascending and transverse colon were labeled as right side tumors. Tumors of the descending and sigmoid colon (without any rectal involvement) were labeled as left side tumors. Tumors extending to parts of sigmoid colon and rectum at the same time are labeled as recto-sigmoid tumors. Tumors involving only the rectum are labeled as rectal tumors.

Association between clinico-pathological features and K-ras status was analyzed using Chi square test. Survival tables and Kaplan Meier test were used for relapse free survival (RFS) and overall survival (OS). SPSS version 12 software was used for all analyses. Approval of institutional review board (ethics committee) has been obtained prior to conducting this study.

Table 1. Patients’ Characteristics and Association between Clinico-pathological Features and K-ras Status

	All (46)	Mutant (15)	Wild (31)	Chi Square
Gender				
Male	30/46 (65%)	13/30 (43%)	17/30 (57%)	RR=1.54 (95% CI: 1.07-2.2) P= 0.034
Female	16/46 (34%)	2/16 (13%)	14/16 (88%)	
Site				P=0.146
Right	17/46 (37%)	8/17 (47%)	9/17 (53%)	
Left	4/46 (9%)	0/4 (0%)	4/4 (100%)	
Recto-sigmoid rectum	17/46 (37%) 8/46 (17%)	6/17 (35%) 1/8 (13%)	11/17 (65%) 7/8 (88%)	
Stage at diagnosis				P=0.60
Stage II	14/46 (30%)	4/14 (31%)	10/13 (77%)	
Stage III	15/46 (33%)	6/15 (43%)	9/14 (64%)	
Stage IV	17/46 (37%)	5/17 (26%)	14/19 (74%)	
Differentiation				P=0.11 Excluding the single WD tumor
Well (WD)	1/46 (2%)	0/1 (0%)	1/1 (100%)	
Moderate Poor	38/46 (83%) 7/46 (15%)	15/38 (39%) 0/7 (0%)	23/38 (61%) 7/7 (100%)	
Vascular invasion				P=0.67
Yes	10/46 (22%)	4/10 (40%)	6/10 (60%)	
No Not known	34/46 (74%) 2/46 (4%)	9/34 (26%)	25/34 (74%)	
Lymph invasion				P=0.78
Yes	9/46 (20%)	3/9 (33%)	6/9 (67%)	
No Not known	35/46 (76%) 2/46 (4%)	10/35 (29%)	25/35 (71%)	

RESULTS

Thirty patients were males and 16 were females. Median age was 61 (21-80) years. Patients' characteristics are depicted in Table 1.

K-ras mutation was identified in 15/46 (32%) tumors. Mutations were identified in codons 12 and 13 in 13/15 (87%), 2/15 (13%) respectively.

Among males, 43% and 57% of tumors were mutant and wild respectively. Among females, 13% and 88% of tumors were mutant and wild respectively.

Gender, site of tumor, stage at diagnosis, tumor differentiation and lymphatic and vascular invasion were tested as potential risk predictors for K-ras status (Table 1). Gender was found to be a potential risk factor. Female gender compared to male posed higher significant chance of wild type status (RR=1.54, 65% CI: 1.07-2.2; P=0.034). There was a trend for relatively higher frequency of mutant status in right side tumors (47%) compared to other sites. However, this did not translate to statistical significance. Site of tumor, stage at diagnosis, tumor differentiation and lymphatic and vascular invasion were not statistically significant predictors for K-ras status.

Median duration of follow up for all patients was 17 (1-67) months.

In mutant and wild type groups, similar proportion of patients experienced relapse or metastatic disease at time of diagnosis (80% & 81% respectively). For patients diagnosed with non metastatic disease (stage II/III, n=29), median RFS was 13.5 (4.7-22.2) months. RFS was not statistically different between mutant & wild type groups (17 vs. 11 months respectively; P=0.256) (Fig. 1). Median OS was not reached in both groups and was not statistically different (P=0.59)

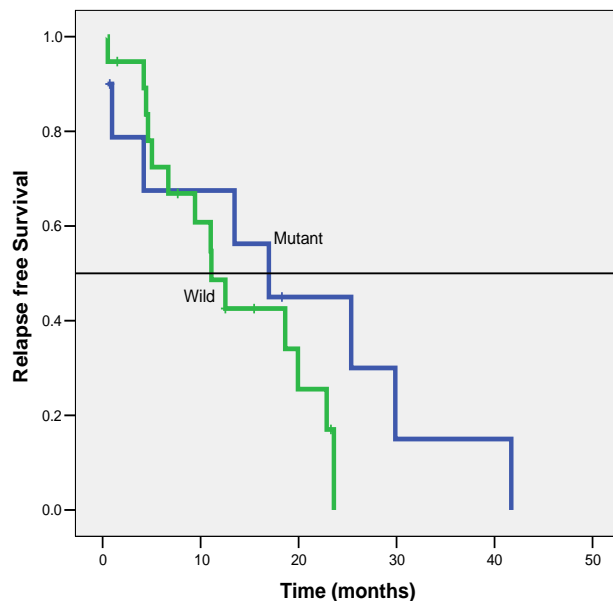


Fig. (1). Relapse Free Survival of Patients with Mutant (n=10) and Wild (n=19) Type K-ras. (Median RFS: M=17 & W=11 months; P=0.256)

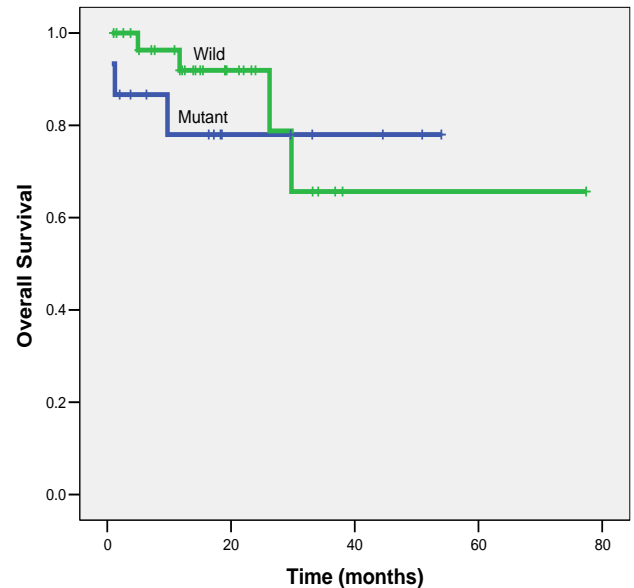


Fig. (2). Overall Survival of Patients with Mutant (n=15) and Wild (n=31) Type K-ras. (Median OS: Not reached for both groups; P=0.59)

(Fig. 2).

DISCUSSION

This study showed that the rate of K-ras mutation exclusively in Saudi patients with stage II/III/IV CRC is 32%. This rate of K-ras mutation in this ethnic group is lower than reported in 4 large randomized trials testing the efficacy of cetuximab in mostly western patients [10-13] (Table 2). Collectively, the average rate of mutation in these 4 studies was 40% (37%-43%). Similarly, in a recent large series of 2121 patients with metastatic CRC from the United States of America, the rate of K-ras mutation was 42% [14].

Difference in rate of K-ras mutation according to geographic area of residence and ethnic origin is increasingly recognized. Clear difference in frequency of mutation was reported in one of these studies according to geographic area of recruited patients [13]. Patients recruited from Western Europe had the highest frequency of mutation followed by Eastern Europe while those from outside Europe had the lowest (Table 2).

The only other study investigating pattern of K-ras mutation in CRC Saudi patients showed a mutation rate of 28.1% [15]. Even lower rates were reported in 53 Kashmiri patients from India (22.6%) and 59 Egyptian patients (11%) with CRC [16, 17].

These findings strongly suggest that rate of K-ras mutation in CRC is lower in Asian and Middle East population than in western and European population. This may be complicated by the extensive range of ethnic origin groups that live in Asia and the Middle East. Despite the low rates of mutation reported in Asian patients of Saudi and Kashmiri ethnic background [15,16, this study], higher rates in other Asian patients of Chinese, Israeli and Japanese ethnic

Table.2. Frequency of K-ras Mutation in Large Randomized Studies Investigating Cetuximab in Patients with Metastatic CRC

Reference and Treatment	Number of Samples Tested/ Number of Total Patients	Rate of K-ras Mutation (%)	Rate of K-ras Mutation in Geo- graphic Groups (%)
[10] BSC vs. Cet	394/572	42.3	NR
[11] FOLFOX vs. FOLFOX&Cet	315/344	43	NR
[12] XELOX&B vs. XELOX&B&Cet	528/755	39.6	NR
[13] FOLFIRI vs. FOLFIRI&Cet	1063/1198	37	Western Europe 44.7 Eastern Europe 35.8 Outside Europe 19.5

BSC = Best supportive care, B = Bevacizumab, Cet = Cetuximab, FOLFOX = Folinic acid, fluorouracil and oxaliplatin, FOLFIRI = Folinic acid, fluorouracil and irinotecan, XELOX = Capecitabine and oxaliplatin, NR = Not reported.

groups; 34.7%, 44.8% and 42% respectively have been reported [18-20]. This suggests a probable ethnic rather than mere geographic variation.

In our study, mutations were identified in codons 12 and 13 in 87% and 13% respectively. This is similar to what is reported in the randomized phase III study comparing cetuximab and best supportive care in patients mostly from Canada and Australia where mutations were identified in codons 12 and 13 in 86.6% and 13.5% respectively [10]. This is also similar to what is reported in patients from Brazil; 87% of mutations were in codon 12 and 13% in codon 13 [21]. In studies conducted on Asian patients, mutations were identified in codons 12 and 13 in 81.2% and 18.8% in Saudi patients and in 68.3% and 29.3% in Chinese patients [15, 18]. This indicates that codon site of mutation is not widely variable among different geographic and ethnic population.

Due to limited number of patients in our series we decided to study the association of K-ras status with only limited clinico-pathological variables. These were Gender, site of tumor, stage at diagnosis, tumor differentiation and lymphatic and vascular invasion. Our results show that Saudi females with CRC are less likely to have mutant K-ras than males. In other word; males are more likely to have mutant K-ras status than females. There are only few reports that show correlation between gender and rate of K-ras mutation. Similar finding was reported in patients from Brazil; a significant difference in the percentage of mutated K-ras was observed between male and female (41 vs. 35%, $p=0.05$) in a cohort of 989 patients [21]. In contrast, K-ras mutation was higher in females than males (44.7% vs. 28.2%, $P=0.037$) in 118 patients from China with the caveat that gender association was different according to the site of tumor [18]. In those Chinese patients, K-ras mutation frequency in the rectum was higher in female than in male patients (34.0% vs. 11.2%, $P<0.05$) and male patients had a higher mutation rate in the colon than female patients (16.9% vs. 10.6%, $P<0.05$). It should be noted that 44.1% of patients in the Chinese study had rectal cancer while only 17% in our study.

In our study and considering 4 different sites of the colorectal tract, there was a trend for relatively higher frequency of mutant status in right side tumors (47%) compared to oth-

er sites (Table 1). However, this did not translate to statistical significance.

When dividing the colorectal tract to only 2 sites; (I) right: caecum and ascending colon and (II) left: transverse, descending and sigmoid colon and rectum, there is a clear trend of higher frequency of mutation in the right colon (47%) compared to the left colon (24%). This is consistent with the findings in the Chinese and the larger Saudi study: mutation rate is 52% vs. 29% and 41% vs. 25.7% at right and left sites respectively [18, 15]. A small study of 12 patients from Romania also showed a trend for higher frequency of mutation (33.3%) in the proximal colon compared to (16.67%) in distal colon [22].

All other clinico-pathological factors tested in our study were not found to significantly predict or associate with K-ras status. The Chinese study showed similar finding where age, tumor location, tumor differentiation and stage did not show significant association with K-ras status [18]. Consistent with our findings, the larger Saudi study did not show significant association between K-ras status and tumor stage and differentiation [15].

European data of 404 patients from Switzerland showed partly similar findings with lack of association between K-ras status and clinico-pathological parameters, such as gender, age, tumor location, histological type, tumor stage, tumor grade and vascular invasion [23].

Some studies have indicated the importance of K-ras alterations in predicting long-term outcome, while others have failed to show such a relationship.

In our study in mutant and wild type groups, similar proportion of patients experienced relapse or metastatic disease at time of diagnosis (80% & 81% respectively) indicating that K-ras status may not predict clinical aggressiveness of disease. This is also supported by the fact that RFS for the 29 patients presented with early resectable disease (stage II/III) and OS for all patients was not statistically different between mutant and wild type groups. Our results are supported by the findings of a study of 508 cases identified among 1264 patients with stage III colon cancer who enrolled in a randomized adjuvant chemotherapy trial. Patients with mutant and wild type tumors did not experience any difference in

RFS or OS [24]. This is in contrast to the finding in another study. The NO147 study investigated the role of adding cetuximab to adjuvant chemotherapy in 2581 patients with stage III colon cancer. Cetuximab did not improve 3 years RFS or OS. However, it was observed that RFS was longer in wild type group than in mutant group in both conventional and investigational (cetuximab) arms; conventional: 75.8% vs. 67.2%, $P=0.04$ and investigational: 72.3% vs. 64.2%, $P=0.004$ [25]. Limitations of our study in this context are: (I) small number of patients eligible for RFS analysis ($n=29$) (II) there is mix of patients with stage II and III disease (III) some patients did while others did not receive adjuvant treatment (IV) relatively short duration of follow up (median 17 months). Difference in clinical outcome according to K-ras status in patients with metastatic disease is also somewhat controversial. In one study comparing cetuximab with BSC, there was no difference in PFS and OS between mutant and wild type groups in the conventional arm. However, PFS and OS were shorter for the mutant type group in the investigational arm [10]. Similar trends were reported in 2 other studies comparing conventional chemotherapy with and without cetuximab [11, 12]. However, in another study comparing conventional chemotherapy with and without cetuximab, PFS and OS were shorter for mutant group than for wild type group in both treatment arms [13]. We will not be able to assess difference in outcome of patients with metastatic disease in our study due to small number and the fact that they used different treatment strategies.

In relatively small series from Asia including patients with all stages of disease (I-IV), the finding of the Chinese study concurs with our results showing no significant difference in OS between mutant and wild type groups [18]. On the other hand the Saudi study reports shorter OS in mutant group compared to wild type group [15].

CONCLUSION

This relatively small retrospective series shows that rate of K-ras mutation in Saudi patients with CRC is 32% which is lower than reported in western Caucasian population but close to rates reported in neighboring Asian and Middle Eastern population. Mutations are less frequent in females. Rates of codon 12 and 13 mutations are similar to those in other parts of the world. In these patients, K-ras mutation status did not significantly affect clinical outcome. More studies with larger cohorts are needed to establish ethnic and geographic variations in K-ras mutation from patients with CRC.

TRIAL REGISTRATION

This is not a clinical trial. This is a retrospective clinical study. Approval of institutional review board (ethics committee) has been obtained prior to conducting this study.

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None declared.

CONFLICT OF INTEREST

None declared.

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