Frequency and Clinical Impact of KRAS Mutations in Patients with Colorectal Cancer from the Middle East

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Abstract: *Background*: Colorectal cancer (CRC) is a significant healthcare burden worldwide and in the Middle East (ME). KRAS mutation confers resistance to epidermal growth factor receptor (EGFR) inhibitors in the treatment of advanced CRC. Data regarding the rate of KRAS mutation from the ME are scattered and scarce. We aim to collect and review all sizable studies evaluating the frequency of KRAS mutations in CRC patients from the ME.

Method: A Pubmed and Google Scholar search was conducted using keywords including KRAS, K-ras, colorectal cancer and Middle East, along with names of each ME country. Studies including over 90 patients were included in the review.

Result: Eleven studies containing more than 90 patients were identified. Among all eleven studies, KRAS mutation rate ranged from 13 to 56%. Five studies reported KRAS mutation rate in M1 stage either exclusively or as part of subgroup analysis. In these studies, mutations were found in 8-45% of cases. KRAS mutations were associated with female gender, M1 stage and high CEA in 3, 2, and 1 studies respectively.

Conclusion: There is a broad range of variability in KRAS mutation rate reported in different studies from the ME. This may have been due to small number of patients in the studies and lack of centralized testing for KRAS mutations. Larger and more coordinated studies from the ME population are required to ascertain the accuracy of KRAS mutation rate.

Keywords: KRAS mutation, colorectal cancer, Middle East, EGFR Inhibitors.

INTRODUCTION

Colorectal cancer (CRC) is the third and fourth most common cancer in women and men worldwide, respectively [1]. GLOBOCAN reports CRC to be the second and fourth most common cancer in women and men respectively in the Middle East (ME) and North Africa area, representing 7.4% of all diagnosed cancer cases [1].

The past 2 decades have seen improvement in the outcome of patients with advanced CRC mostly due to the introduction of both active new cytotoxic chemotherapeutic agents [2] and novel targeted drugs [3]. Cetuximab and Panitumumab are monoclonal antibodies with high affinity for the epidermal growth factor receptor (EGFR), which is over-expressed in up to 80% of colorectal tumors [4, 5]. These antibodies demonstrated the ability to overcome chemotherapy resistance [6] and improve patients' outcome when combined with fluoropyrimidine based chemotherapy in the first line setting [7, 8]. Development of skin rash has been shown to correlate with response and survival to anti-EGFR treatment [6].

RAS oncogene mutations occur in about half of colonic adenomatous and malignant colorectal tumours [9]. The KRAS gene encodes the KRAS protein that regulates downstream PI3K/AKT and RAF/MEK/ERK signal transduction pathways. Thus influencing cell proliferation, differentiation and apoptosis. Most frequent KRAS mutations are in codons 12 and 13 of exon 2. There is an established relation between wild type (WT) KRAS gene status and clinical benefit from anti-EGFR antibodies. In contrast, there lack of benefit in patients with mutant type (MT) KRAS status [10]. For this reason, only patients with tumors expressing WT KRAS genotype are candidates for anti-EGFR therapy.

Most data on frequency and impact of KRAS status is reported on Western populations [10]. Limited available evidence suggests that the molecular pathology of CRC in ME population may be different as compared to Western patients [11].

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Studies investigating KRAS status in Asian and the ME population are scarce, sporadic, and are extracted from small samples. The aim of this report is to identify and review some of these relevant studies to serve as a reference for future studies from this region. We also aim to review the correlation of KRAS mutation with various clinical, pathologic and prognostic factors in ME and Western patients.

METHODS

Extensive Pubmed and Google Scholar search was conducted using different combinations of keywords including KRAS, K-ras, colorectal cancer and Middle East. The key words were also combined with the name of each ME country. The primary targets were studies with large enough sample size that would allow reasonable interpretation. Pragmatically, this was defined as studies including 90 patients or more. Information on KRAS mutation and clinico-pathological characteristics and clinical outcome were also extracted. In addition, smaller studies were quoted and discussed in this manuscript where relevant.

RESULTS

Eleven studies containing more than 90 patients were identified. The findings of these studies are summarized in Table **1**. Six, 3 and 2 studies included patients with different stages, unreported stage and advanced (M1) stage respectively. Among all eleven

Table 1:

Ref	Country	Number	MT	Codons	MT associated with:	No association with:	Survival outcome
Gumus M 2013 ECO [12]	Turkey	All: 1106 (All M1)	498 (45%)	G12D: 64%	NR	Gender or geographic region	NR
Zekri J 2013 [13]	Saudi Arabia	All: 292 M0: 77 M1: 223	119 (41%)	C12: 90% C13: 10%	M1, high CEA	Gender, age, site and grade	Poor RFS and OS
Abubacker J 2009 [14]	Saudi Arabia	All: 285 M0: 252 M1: 33	80 (28%) 71 (28%) 9 (27%)	C12: 81% C13: 19%	Right side	Age, gender, histology, stage or grade	Poor OS: 2.48 (1.41–4.28)
Baskin Y 2014 [15]	Turkey	All: 220 M0: 108 M1: 112	73 (33%) 35 (32%) 38 (34%)	C12: 68% C13: 18% Others: 14%	Females	Age, site, grade or stage	NR
Bishehsari F 2006 [16]	Iran	182 Stage:NR	68 (37%)	C12: 66% C13: 32%	Females (Trend)	HNPCC, sporadic MSI-H or MSS	NR
Selcukbiricik 2013 [17]	Turkey	All: 172 (All M1)	77 (44%)	C12: 78% C13: 21%	Presence of both lung & liver metastases	Site	No correlation with PFS or OS
Zahrani A 2014 [18]	Saudi Arabia	All: 150 M0: 100 M1: 50	85 (56%)	C12: 87% C13: 13%	C12: Rectum, sigmoid & stage IVb	Gender, geographic region, age or grade	NR
Murtaza BN 2014 [19]	Pakistan	All: 150 M0: 100 M1: 50	20 (13%) 16 (16%) 4 (8%)	C12: 60% C13:35% C31: 5%	Females	Age, histology or site	NR
Elbjeirami W 2012 [20]	Jordan	All: 100 M0: 23 M1: 77	44 (44%)	C12: 91% C13: 9%	None	Gender, age or site	NR
Mehdi I 2014 [21]	Oman	100 Stage:NR	31 (31%)	NR	NR	NR	NR
Marchoudi N 2014 [22]	Morocco	All: 92 Stage:NR	22 (24%)	C12: 82% C13: 18%	None	Age, gender or site	NR

HNPCC: Hereditary Non-Polyposis Colon Cancer; MSI: Microsatellite-Instable Tumours; MSS: Microsatellite-Stable Tumours.
All values are approximated to avoid decimals.

studies, KRAS mutation rate ranged from 13 to 56%. Five studies reported KRAS mutation rate in M1 patients either exclusively or as part of subgroup analysis. In these studies, mutations were found in 8-45% of cases. KRAS mutations were associated with female gender, M1 stage and high CEA in 3, 2, and 1 studies respectively.

DISCUSSION

The KRAS mutation rate in studies done on western populations is approximately 40% [10]. In a large population based study that included 1989 cases with all stages of CRC from western USA, KRAS mutations were detected in 31% of cases [23]. A number of retrospective studies have investigated KRAS mutation rate in CRC patients in the ME. Only a few of these studies have had a large enough sample size to allow meaningful conclusions to be drawn.

Geographic Differences in KRAS Mutation Rates

The effect of geographic area on KRAS mutation rate has been previously observed in a large trial investigating the role of adding Cetuximab to chemotherapy in patients with metastatic CRC [24]. In this trial, patients recruited from Western Europe had the highest frequency of mutation (44.7%) followed by Eastern Europe (35.8%) while those from outside Europe had the lowest (19.5%).

In our review, considering all stages, noticeably lower frequencies of mutation have been reported in Pakistan (13%) [19], Morocco (24%) [22] and one study from Saudi Arabia (28%) [14], while higher frequencies have been reported in other studies from Saudi Arabia (56% & 41%) [13, 18], Turkey (33%, 44% & 45%) [12, 15, 17] and Jordan (44%) [20]. In Oman, a ME country in the Gulf region, the frequency of mutation in 100 patients with unreported stage was 31% [21].

It is interesting to note that the two lowest reported frequencies are from Pakistan, a country on the eastern border of the ME, and Morocco, which is at the western border of the ME. Other smaller studies also suggest that rates of mutation are lower in patients from areas further east of the ME region. For example reported mutation frequencies are 22.6% in Indian Kashmir [25] and 21.8% in Thailand [26].

Israel is a Middle Eastern state with majority Jewish and minority Arab population. The rate of KRAS mutation in Israel was reported at 44.8% in 419 tumour samples [27]. Most mutations (82.4%) were identified within codon 12. The paper was published in Hebrew language precluding further interpretation.

Few other smaller studies have investigated the frequency of KRAS mutation in ME population mostly from Arabic ethnicity. In Iraq, the frequency of mutation in 50 patients (with 96% patients having non metastatic disease) was reported to be 48% [28]. In Egypt, KRAS mutations were detected in 5/47 (11%) of patients with stage II/III non metastatic CRC [29]. Two small studies from 2 countries in the western part of the ME, namely, Tunisia and Morocco, reported mutation frequencies of 16/51 (31.5%), and 24/47 (51%) respectively [30, 31]. All patients in the study from Morocco had metastatic disease.

A comparative study of 247 patients with all stages of CRC from 3 ME countries found that KRAS mutation was less common in patients from Egypt (18.4%) than in patients from Jordan (33.3%) and Turkey (34.2%) [32].

Clinically, KRAS status mainly has a therapeutic impact at the time of diagnosis of metastatic disease (stage M1 or stage IV) requiring systemic therapy. For this reason it is of practical importance to focus on the KRAS status in patients with stage M1 or IV. As a reference, large phase III trials investigating anti-EGFR antibodies in patients with metastatic disease represent the richest and the most reliable source of data on KRAS status. These trials recruited patients mostly from Western countries (Europe and North America). The rate of KRAS mutation was 40% reported in combined analysis of 4 randomized studies investigating the role of Cetuximab in 2,292 mostly western patients [10]. In a similar population, the rate of mutation was 40% (440/1096) and 45% (486/1083) in 2 randomized studies investigating the role of Panitumumab in first and second line settings respectively [8, 33]. Some of the studies in our review investigated KRAS status in a mixed population with different tumour stages while others did not report the stage (Table **1**). In this context, the largest Turkish study (TURKRAS study), including 1106 patients, stands out with all its population having metastatic disease. The frequency of mutation in this study was 45%.

Although the available evidence hints at a possible disparity in KRAS mutation status within different areas of the ME, given the relatively small number of patients in the studies and lack of centralized testing of mutation status, along with non-availability of disease stage in

most cases, a definite conclusion of the effect of geography on KRAS mutation rates cannot be drawn. Larger and more coordinated studies from this region may help in clarifying and confirming this rather broad range of reported KRAS mutation rate.

The reported codon site of mutation in studies from the ME region show some variation with mutations in codon 12 constituting 60% - 91% of all identified mutations (Table **1**). This may not reflect true variation as much as an effect of small sample sizes in most of these studies. The largest ME study (TURKRAS study) did not report all codon 12 mutation but reported only G12D mutations which were present in 64% of the tumors. The second largest study sample was from Saudi Arabia and reported codon 12 mutations in 90% of tumors [13]. This is similar to what was reported in the randomized phase III study investigating the role of cetuximab in 394 patients with metastatic CRC from Canada and Australia where mutations were identified in codons 12 and 13 in 86.6% and 13.5% respectively [34]. This is also similar to what was reported in a large series of 989 patients from Brazil with unreported stage where 87% of mutations were in codon 12 and 13% in codon 13 [35]. Brazil is the world's fifth largest country both by geographical area and by population. Its territories are extensive occupying about half of South America and about 22% of the American continent. A recently reported very large study of 8234 samples from patients with metastatic CRC from Brazil reported no significant difference in the codons affected by mutation according to geographical regions [36]. The above data suggest that the geographic factor may not have a great impact on the codon site mutations.

KRAS Mutation and Correlation with Clinicopathologic Characteristics

KRAS mutations were associated with female gender, advanced stage and high CEA in 3, 2, and 1 studies in our review respectively (Table **1**). Similar association was reported in the large study of 8234 patients from Brazil. MT KRAS was detected in 34.8% of females and in 32.5% of males (P=0.03). It seems that this small but statistically significant difference was detectable due to large sample size [36]. However, a smaller Brazilian study of 989 patients showed opposite association with MT KRAS more common in males than females (41 versus 35%, p= 0.05) [35]. In a much smaller sample of 46 patients, we found that males are more likely to have mutant KRAS status than females [37]. Association between mutations and male gender was also reported in the Puerto Rican patient population [38].

Elevated serum CEA at greater than 10 times the normal value correlates with a poor prognosis [39]. Zekri *et al* reported an association between KRAS mutations and high CEA at initial diagnosis in 292 patients from Saudi Arabia (Table **1**). The results showed that 44% of patients with MT tumors had serum CEA level > 40 ug/L compared to 29% of patients with WT tumors [13]. This association between CEA and KRAS status is not widely reported. We found only one other report published in 2013 investigating this association in 215 patients with metastatic CRC. The authors concluded that their findings demonstrate for the first time that the presence of a KRAS mutation correlated with high initial serum CEA levels [17].

The above mentioned study also reported an association between KRAS mutation and younger age. Data from ME do not indicate any association between age and KRAS status (Table **1**). Our group investigated tumor characteristics and outcome of 116 young patients (≤ 40 years) with CRC and found that 40.2% had MT tumors [40]. Available data from the Far East has also not shown any association between age and mutation frequency. For example in one Chinese study, KRAS mutation was detected in 42.1% of patients presenting at young age <40 years and in 44.4% in older patients (P=0.73) [41].

Correlation between KRAS Mutation Status and Prognosis

Early clinical trials investigating the role of anti-EGFR antibodies confirmed the clinical benefit of combining these antibodies with chemotherapy in patients with metastatic CRC. A meta-analysis of seven randomized controlled clinical trials showed that cetuximab and chemotherapy improves OS, PFS, and response rates compared to chemotherapy alone. However, in this meta-analysis cetuximab-related effect was not adjusted for the K-ras tumor status [42].

The realization of role of KRAS mutation in conferring resistance to anti-EGFR antibodies prompted investigators to retrospectively assess the clinical benefit of these antibodies using KRAS as a predictive factors. Meta-analysis of these trials confirmed that the clinical benefit was restricted to patients with wild KRAS [10].

Subsequent clinical trial investigating anti-EGFR antibodies included only patients with wild type KRAS [43, 44].

Despite specific side effects of anti-EGFR antibodies (mostly rash and diarrhea), they do not impact negatively on quality of life [45, 46].

The most common KRAS mutations in codons 12 and 13 are activation mutations, leading to continuous activation of downstream pathways [47]. Results of studies correlating KRAS mutation status with survival have been conflicting. Among 1989 cases of mostly early CRC (only 12% had distant disease) from Washington (USA), KRAS mutation was associated with poorer disease specific survival [23]. Similar association was also reported in 2478 patients with BRAF WT and KRAS MT stage III CRC in the phase III adjuvant cetuximab N0147 trial [48]. Among the KRAS related studies in the ME, the largest study (TURKRAS study) did not report survival outcome [12]. However, the second and third largest studies reported worse outcome in patients with MT compared to those with WT KRAS tumors [13, 14]. The former study showed that 64.4% of patients with MT KRAS were alive at 5 years compared to 78.2% of those with WT KRAS [14]. In the latter study, patients presenting with early stage disease (stages I-III) and MT KRAS had higher relapse rate (58% vs. 45%) and shorter median relapse free survival (RFS) (22 vs. 29 months). Patients presenting with advanced stage disease (stage IV) and MT KRAS had higher mortality rate (34% vs. 18.5%) and shorter median overall survival (OS) (23.5 months vs. not reached). In addition, MT KRAS was an independent risk factor for poor OS [13]. KRAS Mutations were significantly associated with advanced Dukes' stage and positive lymph node status in 53 patients from India and 83 patients from Saudi Arabia [25].

Several reports from other geographical regions also suggest that KRAS mutation may be associated with poor prognosis and advanced disease. For example KRAS mutations correlated with regional lymph node metastasis and tumor stages in 88 patients from Sweden and 99 patients from Japan [49]. In a study of 501 Puerto Rican patients, mutation were detected more frequently in patients with distant metastases compared to those without (50% vs. 37.4%; P=0.020) [38]. Another series of 254 Japanese patients also reported a significant association between poor RFS and MT KRAS [50].

In contrast, KRAS status did not have a major prognostic value on RFS or OS in 1404 patients from the PETACC-3, EORTC 40993, SAKK 60-00 trial [51] and in 508 patients from the CALGB 89803 [52]. The mutation status of the KRAS was not significantly associated with overall survival in the group of patients receiving best supportive care (BSC) alone in a randomized phase III trial comparing cetuximab with BSC [34].

The prognostic value of KRAS status remains controversial. There is some data suggesting that KRAS mutation status is a poor prognostic factor in patients with early stage CRC but not in advanced stage disease [23, 53]. Another plausible explanation to these inconsistent results is that different mutations within different codons can lead to variation in the biological activity of KRAS proteins. Certainly, the large RASCAL II study investigated this notion in 3439 patients and found that among 12 possible mutations on codons 12 and 13, only one mutation on codon 12 (glycine to valine) had a statistically significant impact on RFS and OS. To sophisticate the issue, this mutation appeared to have a greater impact on outcome in Dukes' C than in Dukes' B tumors [54].

Mutations at codons 12 and 13 of exon 2 of KRAS are predictive of lack of response to anti-EGFR therapy in advanced stage disease [10]. Recently, KRAS mutations at codons 59 and 61 of exon 3 and codons 117 and 146 of exon 4 have been identified in 6% and 9% of tumours from patients with advanced CRC respectively. In addition, recently, mutations at exon 2, 3 and 4 have also been identified in 7, 5 and 1% of these patients respectively. These additional KRAS and NRAS mutations have also been found to confer resistance to anti-EGFR therapy [55].

CONCLUSION

There seems to be modest variation in the frequency of KRAS mutation between the ME and the Western hemisphere. However, this interpretation may be limited by the relatively scarce data from the ME. Larger collaborative multi-centre studies from a wider geographical areas in the ME investigating extended RAS (KRAS and NRAS) status and other novel markers are needed. This is best achieved by including patients from the ME in large therapeutic international trials with translational components to investigate existing and novel biomarkers.

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AUTHOR CONTRIBUTIONS

Jamal Zekri and Syed Karim conducted literature review, data extraction from the literature and wrote the manuscript. Ahmed Al-Shehri, Mervat Mahrous, Tarek Darvish and Hani El-Taani reviewed the extracted data and contributed to the final editing of the manuscript.

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest.

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