Biomedical
Research & Therapy

ISSN: 2198-4093
www.bmrat.org

Review

The prevalence of *KRAS* mutation in colorectal cancer patients in Iranian population: A systematic review and meta-analysis study

Mehrdad Payandeh[1], Nasrin Amirifard[2], Masoud Sadeghi[3], Babak Shazad[4], Negin Farshchian[2], Edris Sadeghi[1,5,*], Malihe Dayani[2]

[1]Department of Bone Marrow Transplantation, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

[2]Department of Radiation Oncology, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

[3]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

[4]Department of Internal Medicine, Taleghani Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

[5]Department of Nursing, Borujerd Branch, Islamic Azad University, Borujerd, Iran

***For correspondence:**

sadeghi_mkn@yahoo.com

Competing interests: The authors declare that no competing interests exist.

Received: 07 September 2017
Accepted: 02 October 2017
Published: 16 October 2017

Abstract

Colorectal cancer (CRC) is one of the most common cancers in the World that KRAS mutations are considered as a key step in the progression of CRC. This meta-analysis study aimed to evaluate the prevalence of *KRAS* mutations in CRC patients in Iran. Six online databases including PubMed, Science direct, Scopus, Web of science, Cochran Library, and Scientific Information Database (SID) were searched systematically up to January 2017. A random-effects meta-analysis was used to calculate the estimation of the prevalence of KRAS mutations in CRC patients by the event rate (ER) with 95% confidence interval (95%CI). Out of 82 articles identified from the search, eleven studies included and analyzed for meta-analysis study. The studies included 1814 CRC patients that mean age of the patients was 57.5 years. The pooled ER of the studies for estimation of the prevalence of KRAS mutation in CRC patients was 32.8% (95%CI=28.7-37.3%). The pooled ER of the studies for the prevalence of codon 12 mutation was 72.5% (95%CI=59.8-82.3%) and for codon 13 mutation was 20% (95%CI=14.6-26.7%). The results showed that the prevalence of KRAS mutation in Iran was different with more studies that therefore the geographical area and race can impact on the prevalence of *KRAS* mutation in CRC patients. Also, codon 12 had the most prevalence among mutant codons, followed by codon 13 that Gly to Asp and Gly to Val were the most mutations in codon 12.

Biomedical
Research & Therapy

ISSN: 2198-4093
www.bmrat.org

Keywords

CRC, Iran, KRAS, prevalence

Introduction

Colorectal cancer (CRC) is the fourth most common cancer in men and the third most common in women (Amirifard et al., 2016). There are very few studies about *KRAS* mutations in CRC from developing countries such as Iran (Bishehsari et al., 2006). The latest data from the cancer patients' registry program showed that the age-standardized incidence rate had risen from 2.8 to 5.5 in 2009 and reached 9.2 in 2012 per 100,000 persons (Dolatkhah et al., 2016). *KRAS* is a proto-oncogene located on the short arm of chromosome 12, encodes the protein *KRAS*, a GTPase involved in cell division, differentiation and apoptosis (Dobre et al., 2015). The prevalence of *KRAS* mutation in CRC patients is 35–40 %, and the majority of these mutations occur in codon 12 and less frequently in codon 13 of *KRAS* gene (Rosty et al., 2013). Mutations activating the *KRAS* proto-oncogene are considered a key step in the progression from normal colorectal epithelium to carcinoma (Fearon and Vogelstein, 1990). Roughly 90% of the activating mutations, that are influential solitary amino acid replacement in the GTPase pocket that guide to a block of the activity of *KRAS* p21 protein, are recognized in codons 12 (GGT) and 13 (GGC) of exon 1 and almost 5% in codon 61 (CAA) situated in exon 2. The most regularly found kinds of mutations are G>A and G>T transitions (Palmirotta et al., 2009). This meta-analysis study aimed to indicate the prevalence of *KRAS* mutations in CRC patients in Iran.

Materials and Methods

Search Strategy

Six online databases including PubMed, Science direct, Scopus, Web of science, Cochran Library, and Scientific Information Database (SID) were searched systematically up to January 2017 with the terms of "KRAS" or "K-ras" and "colorectal" or "colon" or "rectum" or "rectal" in combination with "Iran".

Study selection

One author (E.S) searched the articles and then the second author (M.S) blinded to the first author that if there was any disagreement between two authors, both resolved the problems with two-way conversation. The third author (M.P) did the

ISSN: 2198-4093
www.bmrat.org

final revision. The studies were searched for the assessment of prevalence of the *KRAS* mutations in CRC patients in English abstract.

Data Extraction

Name of the first author, year of publication, Province of the region, number of CRC patients, number of mutations, percentage of male (%), the mean age, number of mutant codons, and number of mutant amino acids of codon 12 were the relevant data extracted from every study.

Statistical analysis

A random-effects meta-analysis was used by Comprehensive Meta-Analysis software version 2.0 (CMA 2.0). The event rate (ER) with 95% confidence interval (95%CI) was calculated for estimation of the prevalence of *KRAS* mutations in CRC patients. Heterogeneity between estimates was assessed by the Q and I^2 statistic that for the Q statistic, heterogeneity was considered for $P<0.1$. P-value (2-sided) <0.05 was considered to be statistically significant in this meta-analysis study. Also, publication bias was assessed through funnel plot analysis with the Begg's and Egger's tests.

Results

From the initial 82 articles identified from the search, after excluding the studies, 18 studies were assessed for eligibility. Then, seven studies were excluded based on reasons. At last, eleven studies included and analyzed for meta-analysis study (**Fig. 1**).

The characteristics of the studies included in the meta-analysis are shown in **Table 1**. All studies are case-control studies published between 2006 and 2016. Six studies were studied from Tehran (Central of Iran) (Bishehsari et al., 2006; Roudbari et al., 2016; Shemirani et al., 2011; Sobhani et al., 2010; Tameshkel et al., 2016; Vakil et al., 2016), one study from Shiraz (Southwest of Iran) (Omidifar et al., 2015), two studies from Kermanshah (Western of Iran) (Amirifard et al., 2016; Payandeh et al., 2016), one study from Tabriz (Northwestern of Iran) (Dolatkhah et al., 2016), and one study from South Khorasan (Eastern of Iran) (Naseri et al., 2016). All studies included 1814 CRC patients that mean age of the patients was 57.5 years. In more studies, the prevalence of CRC in males was more than females (Amirifard et al., 2016; Bishehsari et al., 2006; Dolatkhah et al., 2016; Naseri et al., 2016; Omidifar et al., 2015; Payandeh et al., 2016; Roudbari et al., 2016; Shemirani et al., 2011; Tameshkel et al., 2016; Vakil et al., 2016). Number of mutations in codon 12 and 13 and also mutant amino acid in codon 12 has been shown in **Table 1** that Gly is normal amino acid in codon 12 and can change to other amino acids.

ISSN: 2198-4093
www.bmrat.org

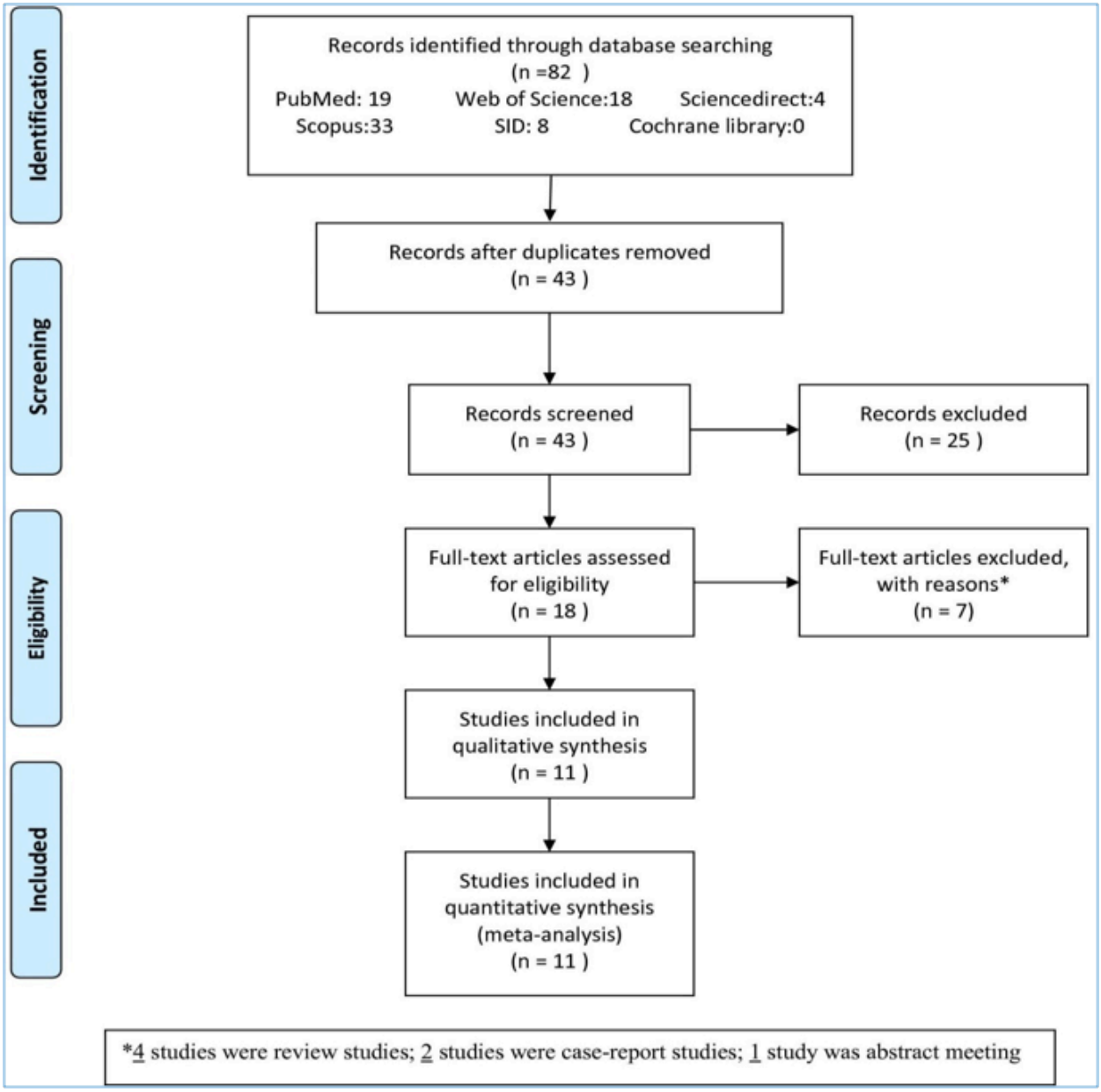

Figure 1. Flowchart of the study.

KRAS mutation

The prevalence of *KRAS* mutation in CRC patients has been reported in **Figure 2** by the ER. The pooled ER of the studies was 32.8% (95%CI=28.7-37.3%) with I^2=58.26% (P=0.008).

Mutant codons

Figure 3 shows the prevalence of mutant codons among all *KRAS* mutations. The pooled ER of the studies for codon 12 was 72.5% (95%CI=59.8-82.3%) with

ISSN: 2198-4093
www.bmrat.org

I^2=83.3% (P<0.001) and for codon 13 was 20% (95%CI=14.6-26.7%) with I^2=54.74% (P=0.015).

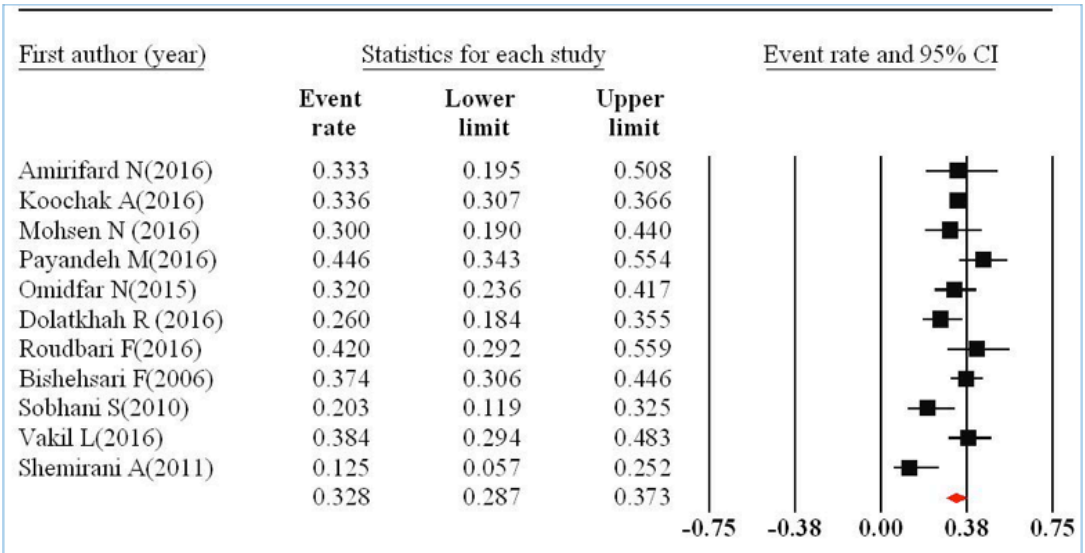

Figure 2. Forest plot of the prevalence of *KRAS* mutation in colorectal cancer patients.

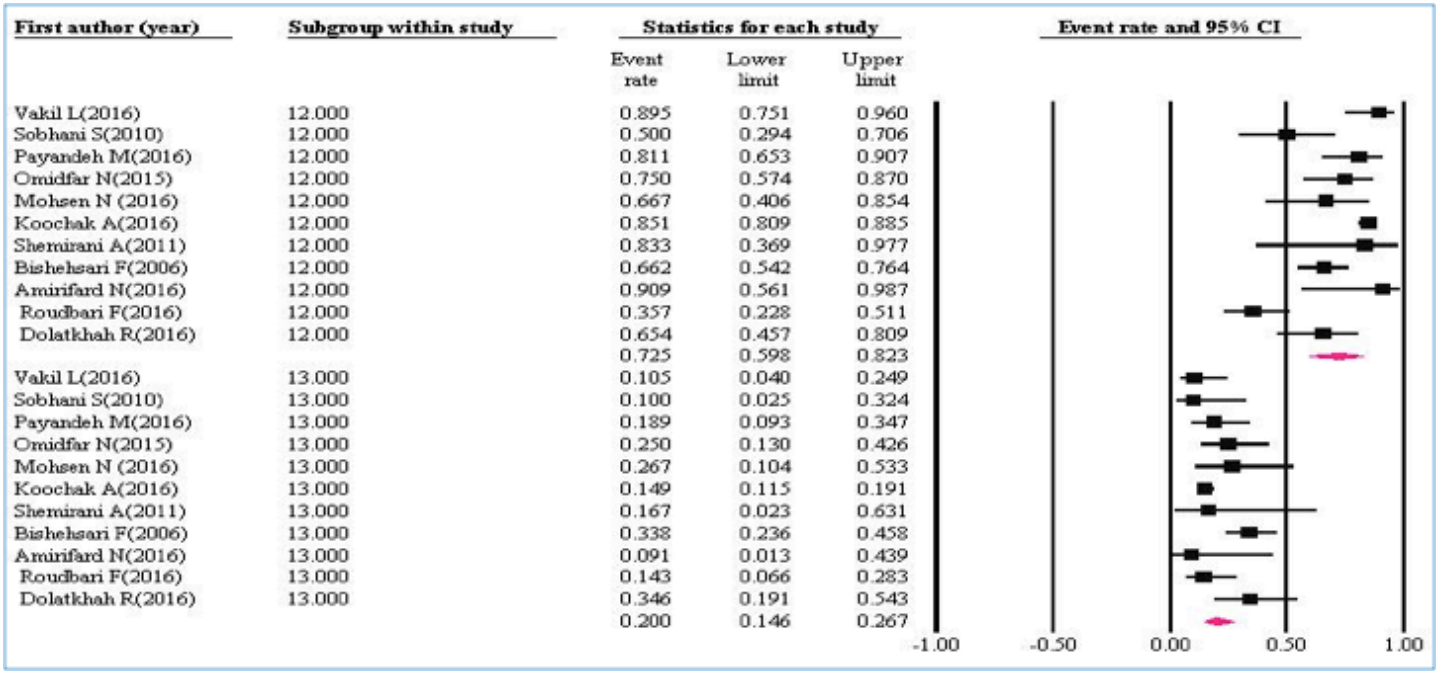

Figure 3. Forest plot of the prevalence of *KRAS* mutant codons in colorectal cancer patients.

ISSN: 2198-4093
www.bmrat.org

Table 1. Characteristics of the studies included in the meta-analysis

First Author (year)	Province	Patients	Total KRAS mutations	Mean age	Sex (%men)	Number of mutant codons	Number of mutant amino acids of codon 12* (n)
Bishehsari et al., 2006	Tehran	182	68	NA	57	12(45), 13(23)	Asp(16), Val(18), Cys(9), Ser(1), Ala(1)
Sobhani et al., 2010	Tehran	59	12	58	41.4	12(10), 13(2)	Asp(6), Ser(2), Val(2)
Shemirani et al., 2011	Tehran	48	6	NA	83	12(5), 13(1)	Asp(4), Cys (1)
Omidifar and Geramizadeh, 2015	Shiraz	100	32	59.1	55	12(24), 13(8)	Ala(12), Asp(9), Ser(1), Cys(1)
Amirifard et al., 2016	Kermanshah	33	11	51.5	78.8	12(10), 13(1)	Asp(5), Val(3), Ser(1), Cys(1)
Dolatkhah et al., 2016	Tabriz	110	26	61.9	65	12(16), 13(9)	Asp(13), Val(2), Cys(1)
Koochak et al., 2016	Tehran	1000	336	55	57.3	12(286), 13(50)	Asp(137), Val(96), Ser(20), Ala(15), Cys(15), Arg(3)
Mohsen et al., 2016	South Khorasan	50	15	60.6	70	12(10), 13(4)	Asp(5), Val(5)
Payandeh et al., 2016	Kermanshah	83	37	57.7	61.4	12(30), 13(7)	Asp(12), Val(8), Ser(4), Ala(2), Cys(3), Arg(1)
Roudbari et al., 2016	Tehran	50	21	56.9	68	12(15), 13(6)	Asp(8), Val(3), Ser(2), Ala(1), Cys(1)
Vakil et al., 2016	Tehran	99	38	57	57.8	12(34), 13(4)	Asp(15), Ala(2), Cys(5), Ser(5), Val(7)

* ***Mutation***: *"Gly" to "other amino acids or mutant amino acid"*

Mutant amino acids of codon 12

Figure 4 shows the prevalence of mutant amino acids among *KRAS* mutations in codon 12. The pooled ER of the studies for "Asp" was 48% (95%CI=41.9-54.2%), "Val" was 30.9% (95%CI=25.3-37.1%), "Ala" was 8.6% (95%CI=2.5-25.6%), "Cys" was 9.9% (95%CI=6-15.9%), and "Ser" was 9.2% (95%CI=6.3-13.2%).

Biomedical
Research & Therapy

ISSN: 2198-4093
www.bmrat.org

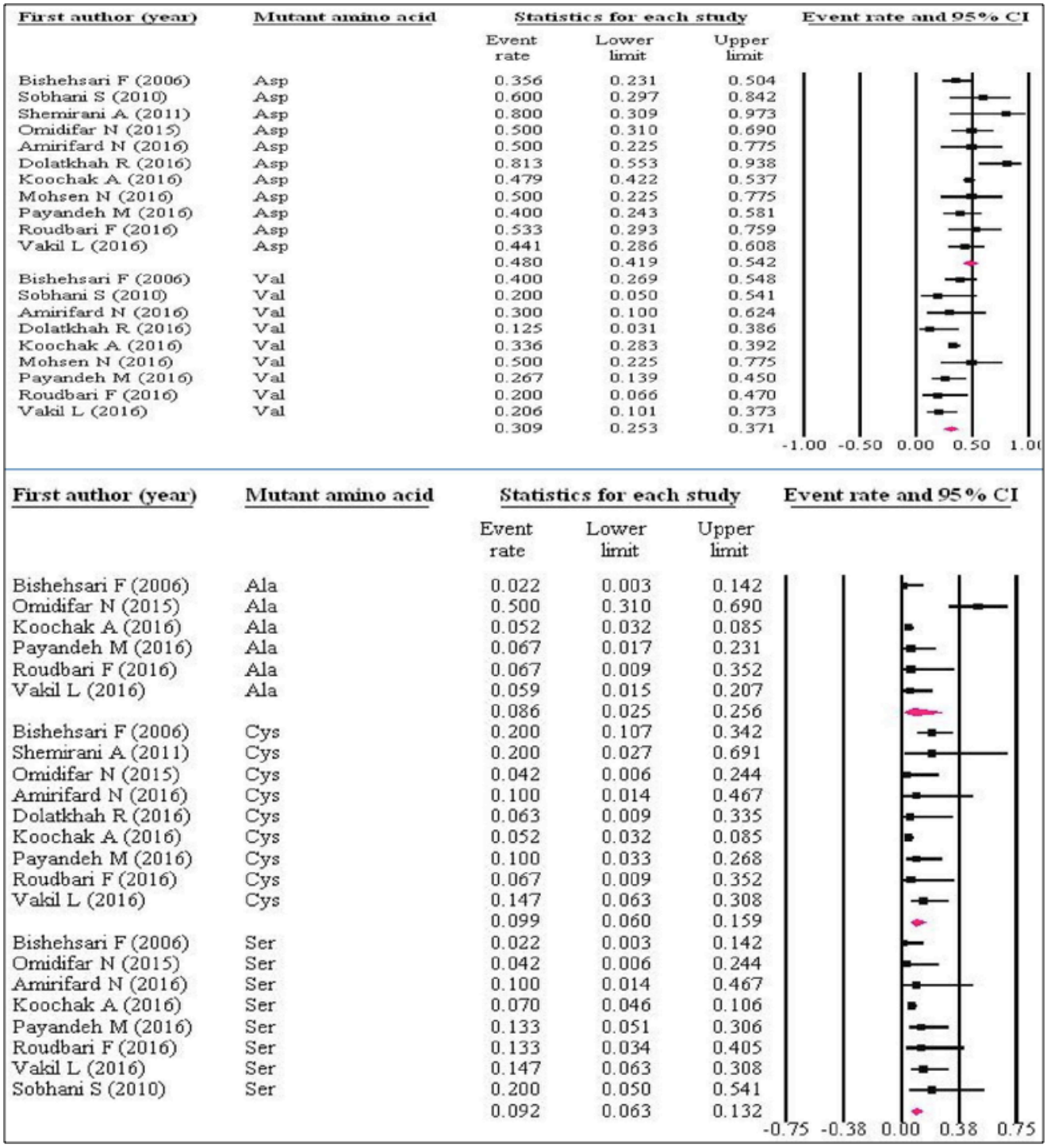

Figure 4. Forest plot of the prevalence of mutant amino acids of codon 12 of *KRAS* mutation in colorectal cancer patients.

ISSN: 2198-4093
www.bmrat.org

Publication bias

Funnel plot of random effect of the studies for the prevalence of *KRAS* mutation in CRC patients has been shown in **Figure 5**. The Begg's and Egger's tests didn't show publication bias (P=0.102 and P=0.433, respectively).

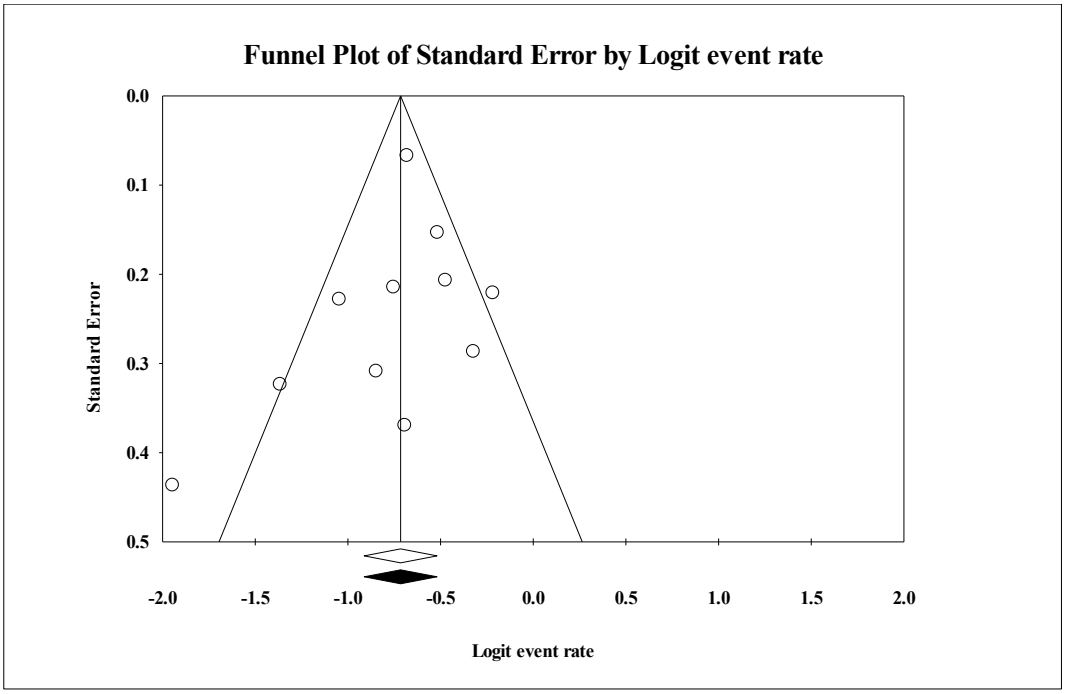

Figure 5. Funnel plot of random effect of the studies for the prevalence of *KRAS* mutation in colorectal cancer patients.

Discussion

The meta-analysis study on eleven studies showed that the ER of *KRAS* mutation was 32.8% (95%CI: 28.7 to 37.3%) in CRC patients in Iran. *KRAS* mutation is one of the most common oncogenic changes in various types of human cancer (Amirifard et al., 2016). Mutation in codons 12, 13, and 61 of *KRAS* is common in CRC (Amirifard et al., 2016). Some studies in different parts of word reported the prevalence of *KRAS* mutation was 24% in the Middle East (Burt, 2000), 36% in Europa, (Ciardiello et al., 2011) 37.4% in Asian, 23% in Thailand, and 50% in Japan. In European countries, *KRAS* mutation was 30% in Italy, 49.3% in the UK, 31.6% in the USA, 28% in Australia, 23 to 28% in Africa, (Omidifar et al., 2015), 38% in France (Chretien et al., 2013) and 62.2% in Italy (Miglio et al., 2013). Therefore, the rate of *KRAS* mutation in the World was between 23 - 50%. Three studies reported in Turkey checked 50, (Gorukmez et al., 2016) 172, (Selcukbiricik et al., 2013) and 53 (Ozen et al., 2013) mCRC patients that the prevalence of *KRAS* mutation was 30%, 44%, and 49.05%, respectively.

ISSN: 2198-4093
www.bmrat.org

Recent reports have shown that *KRAS* mutation in codon 12 is observed at frequencies of 12-30% and 35-50% among CRC patients in Asian and Western countries, respectively (Amirifard et al., 2016). In Romania, *KRAS* mutations in codon 12 accounted for 79.3% and codon 13 was 19.7% (Dobre et al., 2015). In Egyptian, *KRAS* mutations in codons 12 and 13 were present in 90% of cases with mutation (El Kader et al., 2013). One study in France (Chretien et al., 2013) reported *KRAS* mutations in 82.4% happened in codon 12 and 17.6% in codon 13. In sixty-one cases selected from the Greek population, the frequency of the mutation in the codon 12 was 28.3% (Symvoulakis et al., 2007). Mutation status in codon 12 and codon 13 were 62.2% and 21.4% (Miglio et al., 2013). The rate of codons 12 and 13 were 75% and 19%, respectively, that was near to the results of other studies.

In Egyptian, in codon 12, the most regularly found kinds of mutations were Gly to Asp (Dobre et al., 2015). In other study, the most common mutations were transitions of nucleotides Gly to Asp (28.7%) and Gly to Val (25%) (Miglio et al., 2013). The present meta-analysis study showed that Asp (47%) and Val (34%) were the most mutations in Iran that the results were similar.

Limitations

More studies were not sex-matched.

Some studies reported the prevalence just in metastatic CRC and rest of studies both metastatic and non-metastatic CRC.

The studies reported in different areas and races of Iran that geographical area and race can effect on the prevalence of mutations.

In a number of studies, except for codon 12 and 13, types of other different codons have been checked.

Conclusions

The meta-analysis showed that the prevalence of *KRAS* mutation in Iran was different with more studies that therefore the geographical area and race can impact on the prevalence of *KRAS* mutation in CRC patients. Also, Codon 12 had the most prevalence among mutant codons, followed by codon 13. At last, Gly to Asp and Gly to Val were the most mutations in codon 12.

Biomedical
Research & Therapy

ISSN: 2198-4093
www.bmrat.org

Acknowledgment

This article is a part of the research project (code: 95471) supported by a grant from Research Council of Kermanshah University of Medical Sciences.

Abbreviations

CI: Confidence Interval
CRC: Colorectal Cancer
ER: Event Rate
SID: Scientific Information Database

Author Contributions

Conceptualization: MP ES MS
Data curation: ES MS
Formal analysis: ES MS
Funding acquisition: ES MS
Investigation: NA BS NF
Methodology: ES MS
Project administration: MP NA BS NF
Resources: MP MD
Software: ES MS
Supervision: MP NA
Validation: MP ES MS
Visualization: NA BS NF
Writing – original draft: ES MS
Writing – review & editing: MP NA MS BS NF ES MD

ISSN: 2198-4093
www.bmrat.org

References

Amirifard, N., Sadeghi, E., Farshchian, N., Haghparast, A., and Choubsaz, M. (2016). Evaluation of KRAS Gene Mutations in Metastatic Colorectal Cancer Patients in Kermanshah Province. *Asian Pacific journal of cancer prevention: APJCP* 17, 3085-3088.

Bishehsari, F., Mahdavinia, M., Malekzadeh, R., Verginelli, F., Catalano, T., Sotoudeh, M., Bazan, V., Agnese, V., Esposito, D., and De Lellis, L. (2006). Patterns of K-ras mutation in colorectal carcinomas from Iran and Italy (a Gruppo Oncologico dell'Italia Meridionale study): influence of microsatellite instability status and country of origin. *Annals of oncology* 17, vii91-vii96.

Burt, R.W. (2000). Colon cancer screening. *Gastroenterology* 119, 837-853.

Chretien, A.S., Harlé, A., Meyer–Lefebvre, M., Rouyer, M., Husson, M., Ramacci, C., Harter, V., Genin, P., Leroux, A., and Merlin, J.L. (2013). Optimization of routine KRAS mutation PCR–based testing procedure for rational individualized first–line–targeted therapy selection in metastatic colorectal cancer. *Cancer medicine* 2, 11-20.

Ciardiello, F., Tejpar, S., Normanno, N., Mercadante, D., Teague, T., Wohlschlegel, B., and Van Cutsem, E. (2011). Uptake of KRAS mutation testing in patients with metastatic colorectal cancer in Europe, Latin America and Asia. *Targeted oncology* 6, 133.

Dobre, M., Dinu, D.E., Panaitescu, E., Birla, R.D., Iosif, C.-I., Boeriu, M., Constantinoiu, S., Ivan, R.N., Ardeleanu, C.M., and Costache, M. (2015). Kras gene mutations–prognostic factor in colorectal cancer. *Rom J Morphol Embryol* 56, 671-678.

Dolatkhah, R., Somi, M.H., Kermani, I.A., Bonyadi, M., Sepehri, B., Boostani, K., Azadbakht, S., Fotouhi, N., Farassati, F., and Dastgiri, S. (2016). association between proto-oncogene mutations and clinicopathologic characteristics and overall survival in colorectal cancer in east azerbaijan, iran. *OncoTargets and therapy* 9, 7385.

El Kader, Y.A., Emera, G., Safwat, E., Kassem, H.A., and Kassem, N.M. (2013). The KRAS StripAssay for detection of KRAS mutation in Egyptian patients with colorectal cancer (CRC): A pilot study. *Journal of the Egyptian National Cancer Institute* 25, 37-41.

Fearon, E.R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* 61, 759-767.

Miglio, U., Mezzapelle, R., Paganotti, A., Allegrini, S., Veggiani, C., Antona, J., Gentilli, S., Monga, G., Alabiso, O., and Boldorini, R. (2013). Mutation analysis of KRAS in primary colorectal cancer and matched metastases by means of highly sensitivity molecular assay. *Pathology-Research and Practice* 209, 233-236.

Naseri, M., Sebzari, A., Haghighi, F., Hajipoor, F., and Razavi, F.E. (2016). Frequency of K-RAS and N-RAS Gene Mutations in Colorectal Cancers in Southeastern Iran. *Asian Pacific Journal of Cancer Prevention* 17, 4511-4515.

Omidifar, N., Geramizadeh, B., and Mirzai, M. (2015). K-ras mutation in colorectal cancer, a report from southern Iran. *Iranian journal of medical sciences* 40, 454.

Ozen, F., Ozdemir, S., Zemheri, E., Hacimuto, G., Silan, F., and Ozdemir, O. (2013). The proto-oncogene KRAS and BRAF profiles and some clinical characteristics in colorectal cancer in the Turkish population. *Genetic testing and molecular biomarkers* 17, 135-139.

Palmirotta, R., Savonarola, A., Formica, V., Ludovici, G., Del Monte, G., Roselli, M., and Guadagni, F. (2009). A novel K-ras mutation in colorectal cancer. A case report and literature review. *Anticancer research* 29, 3369-3374.

ISSN: 2198-4093
www.bmrat.org

Payandeh, M., Shazad, B., Sadeghi, M., and Shahbazi, M. (2016). Correlation between RAS test results and prognosis of metastatic colorectal cancer patients: a report from Western Iran. *Asian Pac J Cancer Prev* 17, 1729-1732.

Rosty, C., Young, J.P., Walsh, M.D., Clendenning, M., Walters, R.J., Pearson, S., Pavluk, E., Nagler, B., Pakenas, D., and Jass, J.R. (2013). Colorectal carcinomas with KRAS mutation are associated with distinctive morphological and molecular features. *Modern Pathology* 26, 825.

Roudbari, F., Poopak, B., Sheikhsofla, F., and Ghadiani, M. (2016). Evaluation of frequency of kirsten rat sarcoma gene mutations in Iranian colorectal cancer. *Tehran University Medical Journal TUMS Publications* 74, 392-399.

Selcukbiricik, F., Erdamar, S., Ozkurt, C., Molinas Mandel, N., Demirelli, F., Ozguroglu, M., Tural, D., Buyukunal, E., and Serdengecti, S. (2013). The role of K-RAS and B-RAF mutations as biomarkers in metastatic colorectal cancer. *J BUON* 18, 116-123.

Shemirani, A.I., Haghighi, M.M., Milanizadeh, S., Taleghani, M.Y., Fatemi, S.R., Damavand, B., Akbari, Z., and Zali, M.R. (2011). The role of kras mutations and MSI status in diagnosis of colorectal cancer. *Gastroenterology and Hepatology from bed to bench* 4, 70.

Sobhani, S., GHAFFARPOUR, M., MOSTAKHDEMIN, H.Z., Kamali, F., NOUR, M.Z., and HOUSHMAND, M. (2010). The prevalence of common mutation frequency in K-ras codons 12, 13 in Iranian Colorectal Cancer patients.

Symvoulakis, E., Zaravinos, A., Panutsopulos, D., Zoras, O., Papalambros, E., Sigala, F., and Spandidos, D. (2007). Highly conserved sequence of exon 15 BRAF gene and KRAS codon 12 mutation among Greek patients with colorectal cancer. *International Journal of Biological Markers* 22, 12.

Tameshkel, F.S., Sohrabi, M.R., Reza, M., Babaee, H.R., Bahar, B., Imanzade, F., Zamani, F., Khonsari, M.R., Ajdarkosh, H., and Hemmasi, G. (2016). Mutation analysis of KRAS and BRAF genes in metastatic colorectal cancer: a first large scale study from Iran. *Asian Pacific Journal of Cancer Prevention* 17, 603-608.

Vakil, L., Najafipour, R., Rakhshani, N., Zamani, F., Morakabati, A., and Javadi, A. (2016). Investigation of FIH-1 and SOCS3 expression in KRAS mutant and wild-type patients with colorectal cancer. *Tumor Biology* 37, 8841-8848.