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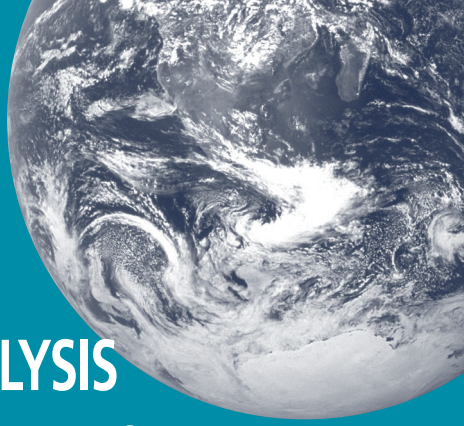

A SYSTEMATIC REVIEW AND META-ANALYSIS ON THE PREVALENCE OF KRAS GENE MUTATION IN SAMPLES OF COLORECTAL CANCER

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Abstract – *Objective:* *Mutation in KRAS gene is one of the most common genetic changes among patients with colorectal cancer (CRC), which is observed in 30-45% of cases. This study aims to estimate the prevalence of this mutation among patients with primary or metastatic CRC.*

Patients and Methods: *Eligible studies were identified during a comprehensive electronic search, applying inclusion/exclusion criteria and quality assessment. Stata version 11 software was used for data analysis. The heterogeneity between the results of the primary studies was assessed using Cochrane and I-square indices. Random effect model was applied for combining the primary estimates. Point and pooled estimates (with 95% confidence intervals) were presented by forest plots. Investigating the factors associated with heterogeneity was carried out using meta-regression models. The publication bias was traced by Egger test.*

Results: *Combining the results of 164 eligible studies, the total prevalence of KRAS gene mutation among primary tumor samples was estimated as of 33.14% (95% confidence interval: 30.08-36.20). The corresponding figure for metastatic cancer was estimated as of 36.20 % (95% confidence interval: 33.96- 38.44). Prevalence of this mutation among patients with primary CRC in EMRO, EURO, PAHO, SEARO and WAPRO was 30.23%, 35.12%, 31.83%, 33.17% and 32.64%, respectively. Corresponding rates for mutation among metastatic cases were 42.20%, 38.46%, 36.06%, 42.80%, 33.05%, respectively. In addition, the total prevalence of KRAS gene mutation in codons 12 and 13 was estimated as 76.69% and 28.49%, respectively.*

Conclusions: *More than one-third of patients with CRC carried KRAS gene mutation particularly in the metastatic tumors. The rate of mutation was the same in different WHO regions.*

KEYWORDS: *Colon cancer, Rectum cancer, Colorectal cancer, CRC, Mutation.*

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INTRODUCTION

Today, cancer is the main cause of mortality leading to 8.8 million deaths per day. According to the WHO reports, lung cancer, breast cancer and CRC are responsible for most of deaths worldwide[1]. Alizadeh-Navaei et al[2] in Iran reported that the highest age-standardized incidence rate of cancers related to women and men were in Yazd Province with 173 and in Markazi Province with 180 cases per 100,000 population, respectively. CRC has led to 774000 deaths and it is considered as the third top cause of mortality[3]. This cancer is the most common malignancy of gastrointestinal system. It is among the most five fatal cancers in men (gastric, prostate, bladder, lung, colorectal cancers) and among the third most fatal cancers in women (breast, gastric and colorectal cancers)[3]. CRC includes tumors of the cecum, ascending/descending/transverse colon, sigmoid and rectum[4]. Based on the results of the recent studies, the incidence of CRC has been increased as 2-4 folds in Asian countries[5]. Mohammadpour et al[6] investigated the prevalence of elevated microsatellite alterations at selected tetranucleotide phenotype in Iranian, where CRC was reported at 41.0%.

Different genetic and environmental mechanisms have been proposed affecting the prognosis and treatment response during CRC. Age over 50, food regimen, smoking and physical activity are environmental risk factors[7]. From the viewpoint of genetic mechanisms, converting the normal epithelium to duplicative epithelium and adenoma is in combination with DNA methylation. There is mutation in KRAS gene during the adenoma stage. However, changing adenoma to carcinoma is in combination with mutation in P53 gene[8].

KRAS gene mutation is one of the most common genetic changes among patients with CRC which is observed in 17-25% of cancers as well as in 30-45% of CRC[9,10]. Mutation in this gene is associated with rapid metastasis, poor prognosis and high invasion of the tumor[11,12]. Most of mutations occur in codons 12 and 13[9,13-16]. Some of the previous studies reported higher rates of KRAS gene mutation among patients with high grades of CRC. Presence of metastasis increases the risk of mutation as of 10%[11]. However, some other studies found that KRAS gene mutation occurred among primary tumors higher than that in metastatic tumors[12].

Several epidemiologic studies have investigated the frequency of KRAS gene mutation during CRC indicating different variations of the gene. Such differences among the results of primary studies can be due to difference in diagnostic methods, geographical situation, genetic and environmental factors, diet, life style and other factors[10,11,15,16]. Meta-analysis is a statistical method which systematically pools the primary results to provide more reliable total estimates. This study aims to estimate prevalence of mutation in KRAS gene among patients with different stages of CRC.

MATERIALS AND METHODS

The protocol of this research has been registered in PROSPERO[17].

Search Strategy

All available databanks such as PubMed, Scopus, Science direct, Ovid and Google scholar were electronically searched and studies carried out during 2000 to 31 August 2016 were identified using the following keywords: "Prevalence", "Frequency", "Percent", "KRAS", "Mutation", Cancer", "Tumor", "Colon", "Rectum", "Colorectal", "CRC".

At least two researchers performed the search. When any disagreement was being observed, another expert was ready to settle the problem. They also investigated each of the studies in term of references to find additional references evidences for meta-analysis.

Selection of the studies

At first, duplicated studies were excluded. Then, titles, abstracts and full texts were reviewed respectively, and irrelevant papers were omitted. In order to find any repeated results, the details of the primary studies were investigated.

Inclusion criteria

The eligible studies should be performed among tumor samples of colon and rectum and diagnosis should be confirmed by pathological report. In addition, sample size and prevalence of mutation in KRAS gene should be reported.

Quality assessment

The selected studies based on the inclusion criteria were assessed using STROBE checklist[18]. Based on this tool, all aspects of methodology were checked. Each study achieved quality score between zero and 44. Studies with total score less than 15.5 (low quality) were excluded from the meta-analysis.

Data extraction

Required information was extracted from each primary study including title, first author name, date of the study, country where the study was conducted, sample size and sampling methodology, type of study samples (primary or metastatic), mean age of

samples, prevalence of KRAS gene mutation among primary and metastatic samples. Excel software was used for collecting data.

Statistical analysis

Standard error of the KRAS gene mutation prevalence was calculated based of binomial distribution equation. The heterogeneity between the findings of the studies was checked according to Cochrane test and I-squared index considering the Higgins criteria[19]. Based on the degree of heterogeneity, random or fixed effect models were applied for combining the primary results. All results (point and pooled prevalence) with 95% confidence intervals were illustrated by forest plots. Publication bias was checked using funnel plot. The source of heterogeneity was investigated using meta-regression models (for variables affecting the heterogeneity) and sensitivity analysis (detecting the role of studies influencing the heterogeneity).

RESULTS

Of 1567 studies identified during databank search, 693 were duplicated. Reviewing titles and abstracts, 576 irrelevant papers were excluded. Full text review revealed 134 irrelevant articles, 129 of which had not reported prevalence of KRAS gene mutation, and five studies were review articles. In addition, quality assessment identified all studies with at least moderate quality. Finally, 164 papers [11,13-16,20-178] were selected for meta-analysis investigating the prevalence of KRAS gene mutation among 37069 subjects with primary CRC and 36477 subjects with metastatic CRC (Figure 1 and Tables 1 and 2).

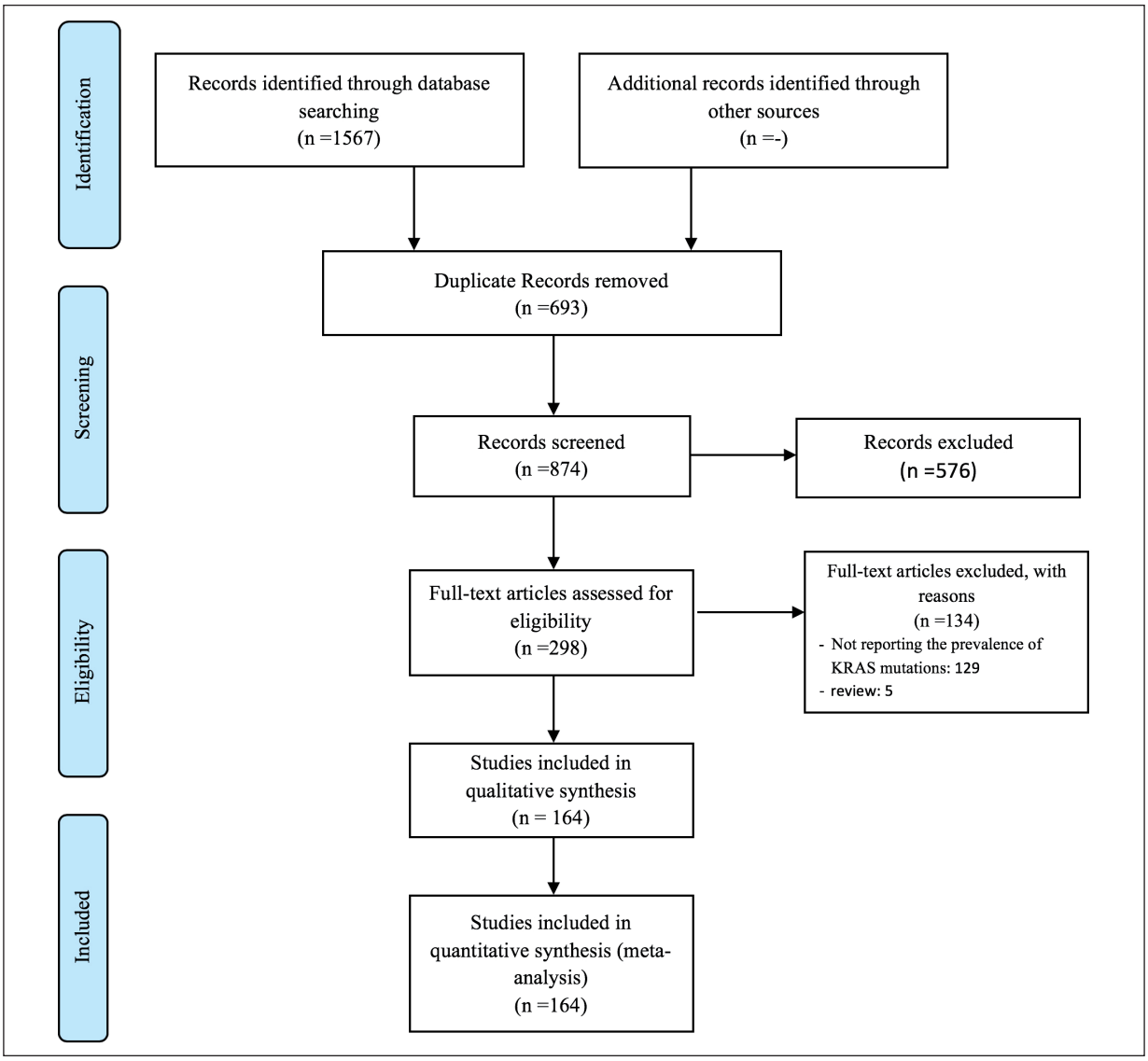

Fig. 1. The process of searching and selecting studies.

TABLE 1. The prevalence of KRAS mutation in primary cancer.

Id	First author	Publication year	Country	Area of WHO	Sample size KRAS in primary	Prevalence primary KRAS	Sample size for codon KRAS	Codon 12 KRAS	Codon 13 KRAS	Codon 12 and 13 KRAS
1	Edkins et al[29]	2006	Hong Kong and USA	WPRO and PAHO	260	4.2	–	–	–	–
2	Bongaerts et al[21]	2006	Netherlands	EURO	4076	4.9	–	–	–	–
3	Weijenberg et al[44]	2008	Netherlands	EURO	4083	5.6	–	–	–	–
4	Miyaki et al[27]	2004	Japan	WPRO	33	6	–	–	–	–
5	Rothschild et al[23]	1997	USA	PAHO	17	6.17	–	–	–	–
6	Irani Shemirani et al[16]	2011	Iran	EMRO	48	12.5	–	–	–	–
7	KA et al[45]	2014	Colombia	PAHO	30	13.3	–	–	–	–
8	Barresi et al[46]	2015	Italy	EURO	175	14	–	–	–	–
9	Egoavil et al[47]	2011	Peru´	PAHO	91	16.7	–	–	–	–
10	Martinetti et al[15]	2014	Albania	EURO	159	17.6	–	–	–	–
11	Lary et al[30]	2011	Iran	EMRO	54	18.5	10	0	100	–
12	Hiraoka et al[48]	2006	Japan	WPRO	205	18.5	–	–	–	–
13	Chang et al[49]	2014	Taiwan	WPRO	94	19.1	–	–	–	–
14	Dolatkhah et al[11]	2015	Iran	EMRO	30	20	–	–	–	–
15	Sobhani et al[14]	2011	Iran	EMRO	59	20.3	–	–	–	–
16	Kwon et al[50]	2011	Korea	WPRO	92	20.7	–	–	–	–
17	Liou et al[51]	2011	Taiwan	WPRO	314	20.7	–	–	–	–
18	Chan et al[52]	2016	Hong Kong	WPRO	69	21.7	–	–	–	–
19	Netzel et al[35]	2013	USA	PAHO	130	22.3	29	–	–	100
20	Ward et al[53]	1998	Australia	WPRO	219	22.4	–	–	–	–
21	Rennert et al[54]	2007	USA	PAHO	212	22.6	–	–	–	–
22	Bleeker et al[55]	2000	Netherlands	EURO	55	23	13	69.2	30.8	–
23	Ito et al[56]	2014	Japan	WPRO	603	23	–	–	–	–
24	Bisht et al[25]	2014	India	SEARO	204	23.5	–	–	–	–
25	Marchoudi et al[57]	2013	Morocco	EMRO	92	23.91	22	81.8	18.2	–
26	Yip et al[58]	2013	Malaysia	WPRO	44	25	–	–	–	–
27	Lee et al[59]	2015	Korea	WPRO	100	26	–	–	–	–
28	Wu et al[34]	2005	Taiwan	WPRO	181	26.5	48	–	–	100
29	Notarnicola et al[60]	2000	Italy	EURO	26	26.9	–	–	–	–
30	Ghosh et al[61]	2013	United Kingdom	EURO	74	27	20	85	–	–
31	Smith et al[62]	2002	Scotland	EURO	106	27.4	–	–	–	–
32	Benedix et al[63]	2012	Germany	EURO	171	27.5	–	–	–	–
33	Andreyev et al[64]	1998	United Kingdom	EURO	2721	27.7	–	–	–	–
34	Naghibalhossaini et al[65]	2011	Iran	EMRO	86	28	–	–	–	–
35	Zhang et al[66]	1998	Sweden	EURO	149	28	–	–	–	–
36	Rosty et al[67]	2013	Australia	WPRO	776	28	–	–	–	–
37	Kamal et al[68]	2012	Egypt	EMRO	80	28.7	–	–	–	–
38	Zhao et al[69]	2008	Japan	WPRO	76	28.9	–	–	–	–
39	Kambara et al[70]	2016	Australia	WPRO	145	29.6	–	–	–	–
40	Ko et al[31]	1998	China	WPRO	99	30	30	100	–	–
41	Palomba et al[71]	2012	Italy	EURO	478	30	–	–	–	–
42	Omidifar et al[13]	2015	Iran	EMRO	100	31	31	74.2	25.8	–
43	Nagasaka et al[72]	2004	Japan	WPRO	234	31	–	–	–	–
44	Phipps et al[73]	2013	New Zealand	WPRO	1923	31	593	75	22	–
45	Aissi et al[74]	2013	France	EURO	51	31.5	16	81.2	18.8	–
46	Zauber et al[75]	2013	Scotland	EURO	171	31.6	–	–	–	–
47	Raskin et al[76]	2013	USA	PAHO	75	32	–	–	–	–
48	Takashi et al[77]	2010	Japan	WPRO	224	32.1	–	–	–	–
49	Baskin et al[78]	2014	Turkey	EURO	94	32.1	27	88.9	11.1	–
50	Yunxia et al[79]	2010	Australia	WPRO	101	32.7	–	–	–	–
51	Hsieh et al[80]	2012	Taiwan	WPRO	182	33	–	–	–	–
52	Nagasaka et al[81]	2008	Japan	WPRO	243	33	–	–	–	–

Continued

TABLE 1 (CONTINUED). The prevalence of KRAS mutation in primary cancer.

Id	First author	Publi-cation year	Country	Area of WHO	Sample size KRAS in primary	Preva-lence primary KRAS	Sample size for codon KRAS	Codon 12 KRAS	Codon 13 KRAS	Codon 12 and 13 KRAS
53	Li et al[82]	2012	China	WPRO	78	33.3	–	–	–	–
54	Nakanishi et al[83]	2012	Japan	WPRO	254	33.5	–	–	–	–
55	Yuen et al[84]	2002	Hong Kong	WPRO	208	33.7	–	–	–	–
56	Wang et al[85]	2003	Australia	WPRO	396	38	–	–	–	–
57	Krol et al[86]	2012	Netherlands	EURO	120	33.9	–	–	–	–
58	Cejas et al[87]	2009	Spain	EURO	110	34	–	–	–	–
59	Boughdady et al[88]	1992	United Kingdom	EURO	29	34.5	–	–	–	–
60	Prall et al[32]	2007	Germany	EURO	95	34.7	32	90.6	6.2	–
61	Shen et al[89]	2011	China	WPRO	118	34.7	41	68.3	29.3	–
62	Sinicrope et al[90]	2014	Switzerland	EURO	2720	34.7	–	–	–	–
63	Negru et al[91]	2013	Greece	EURO	2424	34.9	–	–	–	–
64	Velho et al[92]	2008	Portugal	EURO	17	35.3	–	–	–	–
65	Sakai et al[93]	2015	Japan	WPRO	150	35.3	–	–	–	–
66	Slah et al[94]	2013	Tunisia	EMRO	48	35.41	17	70.6	29.4	–
67	Fariña Sarasqueta et al[95]	2011	Netherlands	EURO	296	36	–	–	–	–
68	Imamura et al[33]	2013	USA	PAHO	451	36	451	74.3	24.4	1.4
69	Brandstedt et al[96]	2014	Sweden	EURO	494	36.4	–	–	–	–
70	Samara et al[97]	2015	Greece	EURO	322	36.6	–	–	–	–
71	Li et al[98]	2015	China	WPRO	945	36.6	–	–	–	–
72	Brink et al[99]	2003	Netherlands	EURO	737	37	–	–	–	–
73	Bishehsar et al[100]	2006	Iran	EMRO	182	37.4	–	–	–	–
74	Gao et al[101]	2012	China	WPRO	966	38.8	–	–	–	–
75	Lin et al[102]	2014	Taiwan	WPRO	1063	38.8	–	–	–	–
76	Hanna et al[103]	2013	Korea	WPRO	83	39	–	–	–	–
77	Kohonen-Corish et al[104]	2014	Australia	WPRO	364	39.3	–	–	–	–
78	Lin et al[105]	2013	Taiwan	WPRO	118	39.8	–	–	–	–
79	Zhu et al[106]	2012	China	WPRO	557	40.4	225	79.1	20.4	–
80	Lee et al[107]	2015	Korea	WPRO	130	40.7	–	–	–	–
81	Chien et al[108]	2006	Taiwan	WPRO	29	41	–	–	–	–
82	Jiang et al[109]	2013	Vietnam	WPRO	61	41	–	–	–	–
83	Baldus et al[110]	2010	Germany	EURO	100	41	–	–	–	–
84	Takashi et al[111]	2001	Japan	WPRO	31	41.9	–	–	–	–
85	Borràs et al[112]	2011	Spain	EURO	93	41.9	–	–	–	–
86	Chiang et al[113]	1997	Taiwan	WPRO	50	42	–	–	–	–
87	Elsabah et al[114]	2013	Egypt	EMRO	26	42.3	–	–	–	–
88	Veldore et al[26]	2014	India	SEARO	299	42.8	–	–	–	–
89	Voutsina et al[115]	2013	Greece	EURO	83	43	–	–	–	–
90	Simi et al[116]	2008	Italy	EURO	116	43.1	–	–	–	–
91	Ahn et al[117]	2014	Korea	WPRO	164	43.3	–	–	–	–
92	Richman et al[42]	2009	London	EURO	711	43.3	–	–	–	–
93	Yan et al1[18]	2015	China	WPRO	122	43.4	–	–	–	–
94	Mao et al[119]	2012	China	WPRO	57	43.9	–	–	–	–
95	Yamashita et al[120]	1995	Japan	WPRO	25	44	–	–	–	–
96	Chen et al[121]	2014	China	WPRO	214	44.9	–	–	–	–
97	Stefanius et al[122]	2010	Finland	EURO	42	45.2	–	–	–	–
98	Kocián et al[123]	2011	Czech Republic	EURO	44	45.5	20	65	35	–
99	Yamane et al[124]	2014	Portugal	EURO	47	46.8	22	100	–	–
100	Sakai et al[28]	2015	Japan	WPRO	100	47	47	–	–	91.4
101	Sclafani et al[125]	2014	Spain	EURO	149	47.6	–	–	–	–
102	Haley et al[126]	2015	USA	PAHO	146	48	–	–	–	–
103	Ozen et al[127]	2012	Turkey	EURO	53	49.5	26	65.4	26.9	–
104	Cushman-Vokoun et al[128]	2013	USA	PAHO	111	49.5	–	–	–	–

Continued

TABLE 1 (CONTINUED). The prevalence of KRAS mutation in primary cancer.

Id	First author	Publication year	Country	Area of WHO	Sample size KRAS in primary	Prevalence primary KRAS	Sample size for codon KRAS	Codon 12 KRAS	Codon 13 KRAS	Codon 12 and 13 KRAS
105	Anker et al[129]	1997	United Kingdom	EURO	14	50	7	100	–	–
106	Senagore et al[130]	1997	USA	PAHO	89	50	–	–	–	–
107	Miles et al131	2016	United Kingdom	EURO	33	51.5	–	–	–	–
108	Cushman et al[24]	2015	USA	PAHO	103	52.4	–	–	–	–
109	Dono et al[132]	2012	Italy	EURO	213	60.1	–	–	–	–
110	Losi et al[22]	1992	Switzerland	EURO	35	71	–	–	–	–
111	El Kader et al[20]	2012	Egypt	EMRO	20	80	16	87.5	–	12.5

A significant heterogeneity was observed among primary studies (I-squared: 98.3%, Q=6553.5, $p<0.001$). Therefore, random effect model was applied for combining the results. The total prevalence of KRAS gene mutation among samples with primary CRC was estimated as of 33.14% (95% confidence interval: 30.80- 36.20) (Figure 2). Meta-regression model showed that WHO region had no effect on the heterogeneity (β=-0.40, $p=0.596$). Funnel plot showed significant publication bias (Figure 3).

Of the selected studies, 12 articles were conducted in EMRO reporting mutation prevalence from 12.5% in Iran (Shemirani) to 80% in Egypt (El Kader)[16, 20]. Combining the results of these 12 studies, the total prevalence of KRAS gene mutation in this region was estimated as of 30.23% (95% confidence interval: 22.94- 37.53).

According to the results of 40 studies carried out in EURO region, prevalence of mutation varied from 4.9% in the Netherland (Bongaerts) to 71% in Swiss (Losi)[21,22]. Combining the primary results, the total prevalence of KRAS gene mutation among patients with primary CRC in this region was estimated as of 35.12% (95% confidence interval: 30.16-40.08).

According to the results of 11 studies carried out in PAHO region, prevalence of mutation varied from 5.17% (Rothschild et al[23]) to 52.40% in the USA (Cushman et al[24]). Combining the primary results, the total prevalence of KRAS gene mutation among patients with primary CRC in this region was estimated as of 31.83 % (95% confidence interval: 23.47-40.20).

In SEARO region two studies were conducted reporting KRAS gene mutation among patients with primary CRC in India from 23.50% (Bisht et al[25]) to 42.80% (Veldore et al[26]). The pooled prevalence of mutation in this region was estimated as of 33.17% (95% confidence interval: 14.25- 52.08).

In WAPRO region 45 primary studies had been carried out regarding KRAS gene mutation prevalence. Minimum and maximum prevalence was reported in Japanese population varied between 6% and 47% in the studies conducted by Miyaki et al[27] and Sakai et al[28], respectively. Combining the primary results, it was showed that the prevalence of the mutation in this region was 32.64% (95% confidence interval: 30.29- 34.98). In addition, another study was conducted by Edkins et al[29] in Hong Kong and in the USA reporting that 4.2% of the samples with primary CRC were KRAS gene mutation carriers.

Prevalence of mutation in KRAS gene in codon 12 was reported in 19 studies varied between zero in a study conducted in Iran by Lary et al[30] to 100% in the study conducted by Ko et al[31] in China. Combining the results of these studies, revealed that total prevalence of this mutation was 76.69% (95% confidence interval: 57.26- 96.12) (Figure 4).

In 14 studies, mutation in KRAS gene in codon 13 was investigated and 13 studies included meta-analysis (the study of Lary et al[30] excluded based on sensitivity analysis). The lowest prevalence of this mutation was 6.2% in the study carried out by Prall et al[32] in Germany. The total prevalence of this mutation was estimated as of 20.81% (95% confidence interval: 16.78- 24.83) (Figure 5).

Mutation in KRAS gene codon 12 and 13 was investigated in five studies (Figure 6). The corresponding prevalence was between 1.4% in the USA (Imamura et al[33]) and 100% in the studies carried out by Wu et al[34] and Netzel et al[35] in Taiwan and the USA, respectively. The total prevalence of this mutation was estimated as of 68.41% (95% confidence interval: 65.28- 71.54).

KRAS gene mutation in the metastatic samples of patients with CRC was investigated in 57 studies (Figure 7). Combining the prevalence reported by these primary studies using random effect model (I-squared=94%, Q=930.44, $p<0.001$), the pooled prevalence of mutation among metastatic samples was 36.20% (95% confidence interval: 33.96- 38.44). Of these 57 studies, 22 were carried out in EURO region reporting prevalence of mutation from 10.10% in Uvirova et al[36] study in Czech to 78% in the study conducted in Denmark[37]. The combined prevalence of mutation in this region was estimated as of 38.46% (95% confidence interval: 34.31-42.61).

TABLE 2. The prevalence of KRAS mutation in metastatic cancer.

Id	First author	Publi-cation year	Country	Area of WHO	Sample size KRAS in metastasis	Prevalence of KRAS in metastasis
1	Giannini et al[133]	2013	Italy	EURO	26	53.8
2	Schweiger et al[134]	2013	Hungary	EURO	39	48
3	Spindler et al[37]	2012	Denmark	EURO	41	78
4	Lin et al[135]	2011	Taiwan	WPRO	42	38.1
5	Phua et al[136]	2015	Singapore	WPRO	45	33.3
6	Gorukmez et al[137]	2016	Turkey	EURO	50	30
7	Fornaro et al[138]	2010	Italy	EURO	52	44
8	Erben et al[139]	2011	Germany	EURO	57	31.6
9	Liao et al[140]	2010	China	WPRO	61	19.7
10	Freeman et al[141]	2008	USA	PAHO	62	38.7
11	Morelli et al[142]	2014	Germany	EURO	62	44
12	Pricolo et al[143]	1996	USA	PAHO	70	36
13	Rako et al[144]	2012	Croatia	EURO	73	35.6
14	Kim et al[145]	2012	Korea	WPRO	82	24.4
15	Bando et al[38]	2013	Japan	WPRO	82	3.6
16	Voutsina et al[115]	2013	Greece	EURO	83	43
17	Bader et al[43]	2014	Saudi Arabia	EMRO	83	42.2
18	Li et al[146]	2013	China	WPRO	87	31
19	Lievre et al[147]	2008	France	EURO	89	27
20	Li et al[148]	2010	China	WPRO	90	33.3
21	Yen et al[149]	2010	China	WPRO	95	43.1
22	Umeda et al[150]	2013	Japan	WPRO	100	27
23	Lahti et al[41]	2015	USA	PAHO	104	43.3
24	Cejas et al[87]	2009	Spain	EURO	110	34
25	Hinoue et al[151]	2012	Netherlands	EURO	125	27
26	Yeh et al[40]	2016	USA	PAHO	126	21
27	Duldulao et al[152]	2013	USA	PAHO	148	41
28	Kemeny et al[153]	2014	USA	PAHO	169	30.2
29	Uvirova et al[36]	2015	Czech Republic	EURO	169	10.1
30	Herreros-Villanueva et al[154]	2011	Spain	EURO	186	48
31	Jakovljevic et al[155]	2012	Serbia	EURO	190	34.7
32	Prenen et al[156]	2009	Belgium	EURO	199	38.7
33	Akiyoshi et al[39]	2013	Japan	WPRO	199	46.2
34	Karagkounis et al[157]	2013	USA	PAHO	202	29
35	Yokota et al[158]	2011	Japan	WPRO	229	34.5
36	Capella et al[159]	1991	USA	PAHO	244	40.1
37	Veldore et al[6]	2014	India	SEARO	299	42.8
38	Arcila et al[160]	2010	USA	PAHO	308	39
39	Sorbye et al[161]	2015	Sweden	EURO	354	51
40	Sasaki et al[162]	2016	Japan	WPRO	378	42.6
41	Lee et al[163]	2014	Korea	WPRO	388	26.5
42	Pereira et al[164]	2014	USA	PAHO	494	41
43	Ogino et al[165]	2009	USA	PAHO	508	35
44	Cuyun Carter et al[166]	2015	USA	PAHO	648	42.3
45	Shen et al[167]	2013	China	WPRO	674	35.9
46	Richman et al[42]	2009	USA	PAHO	711	43.3
47	De Roock et al[168]	2010	Seven Europeancountries (Italy, France, Switzerland,Denmark)	EURO	747	40
48	Kadowaki et al[169]	2014	Japan	WPRO	812	38
49	Maus et al[170]	2013	Germany	EURO	838	39
50	Smith et al[171]	2013	Brazil	PAHO	1076	24
51	Zhang et al[172]	2015	China	WPRO	1110	45.4
52	Pinto et al[173]	2011	Portugal	EURO	1116	41.9
53	Roth et al[174]	2009	Switzerland	EURO	1299	37
54	Vaughn et al[175]	2011	USA	PAHO	2121	42.4
55	Scott et al[176]	2014	Australia	WPRO	3688	38.8
56	Piton et al[177]	2015	France	EURO	6803	30.3
57	Ferreira et al[178]	2014	Brazil	PAHO	8234	31.9

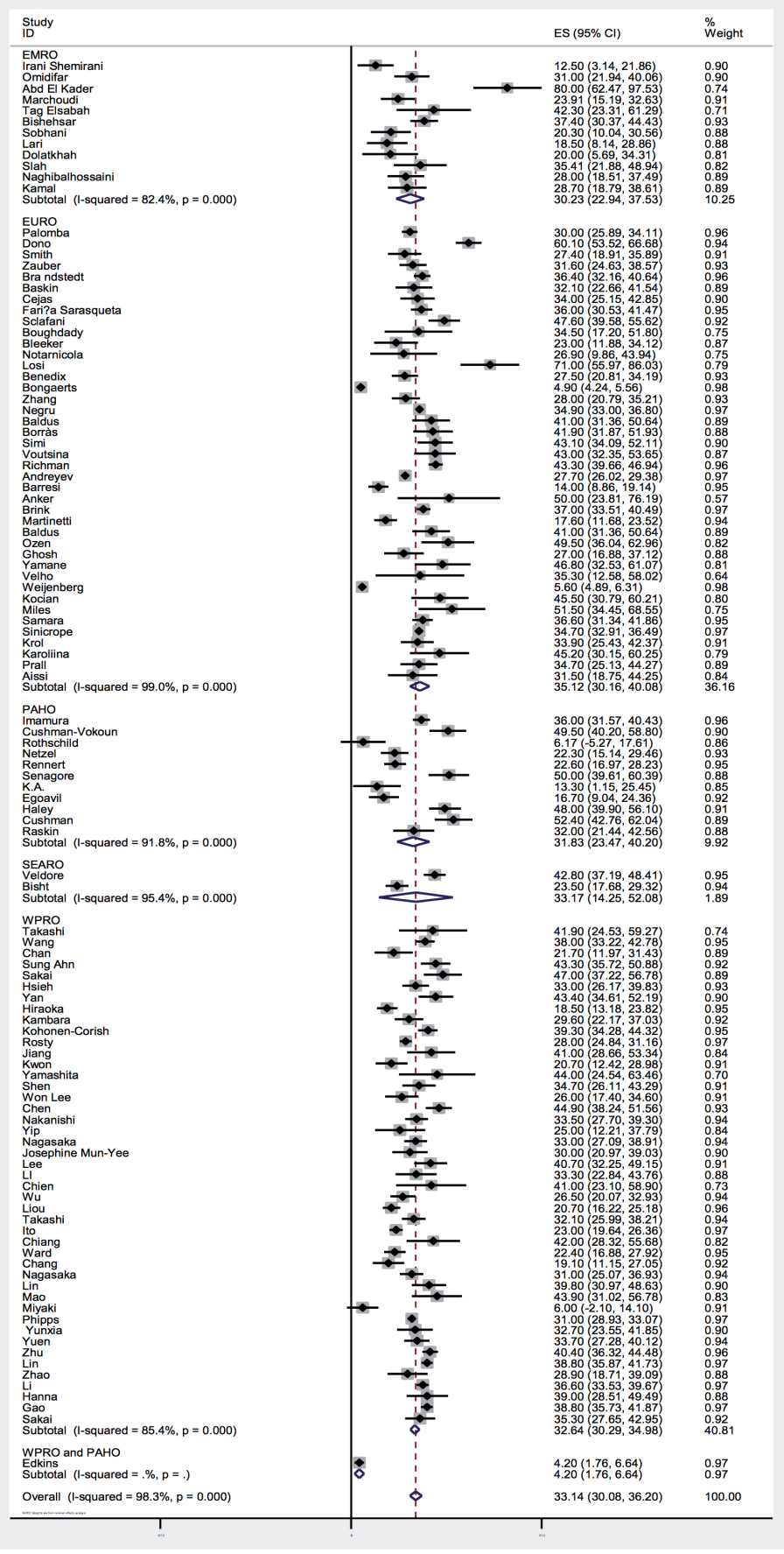

Fig. 2. Point and pooled prevalence of KRAS gene mutation among patients with primary colorectal cancer.

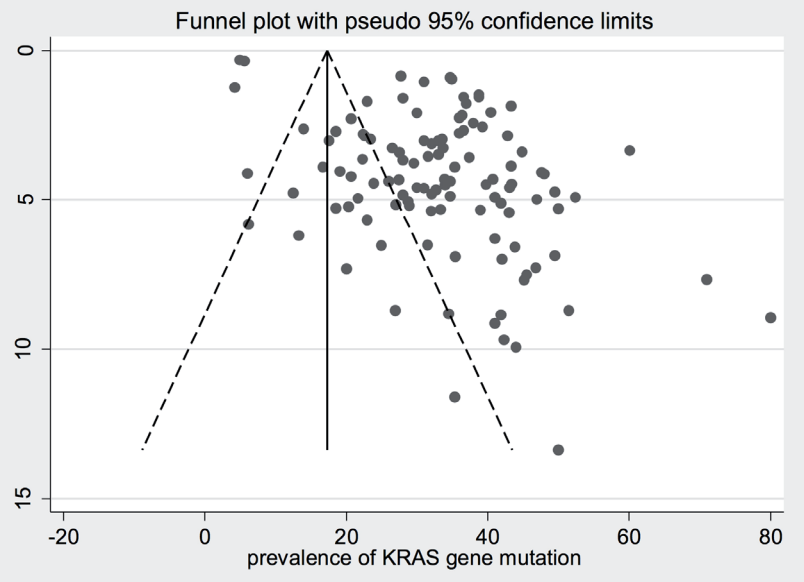

Fig. 3. Funnel plot for investigating publication bias (prevalence of KRAS gene mutation in primary colorectal cancer).

Among 17 studies carried out in WPRO region, prevalence of KRAS gene mutation among metastatic samples varied from 3.60% to 46.2% in the two studies conducted in Japan[38, 39]. The pooled prevalence of this mutation in WPRO region was 35.92% (95% confidence interval: 34.90- 36.94).

Among 16 studies conducted in PAHO region, the prevalence of mutation was reported from 21% to 43.3% in the three studies conducted in the USA[40-42]. The pooled prevalence in the region was estimated as of 33.05 % (95% confidence interval: 27.55- 38.55). It should be noted that just one study

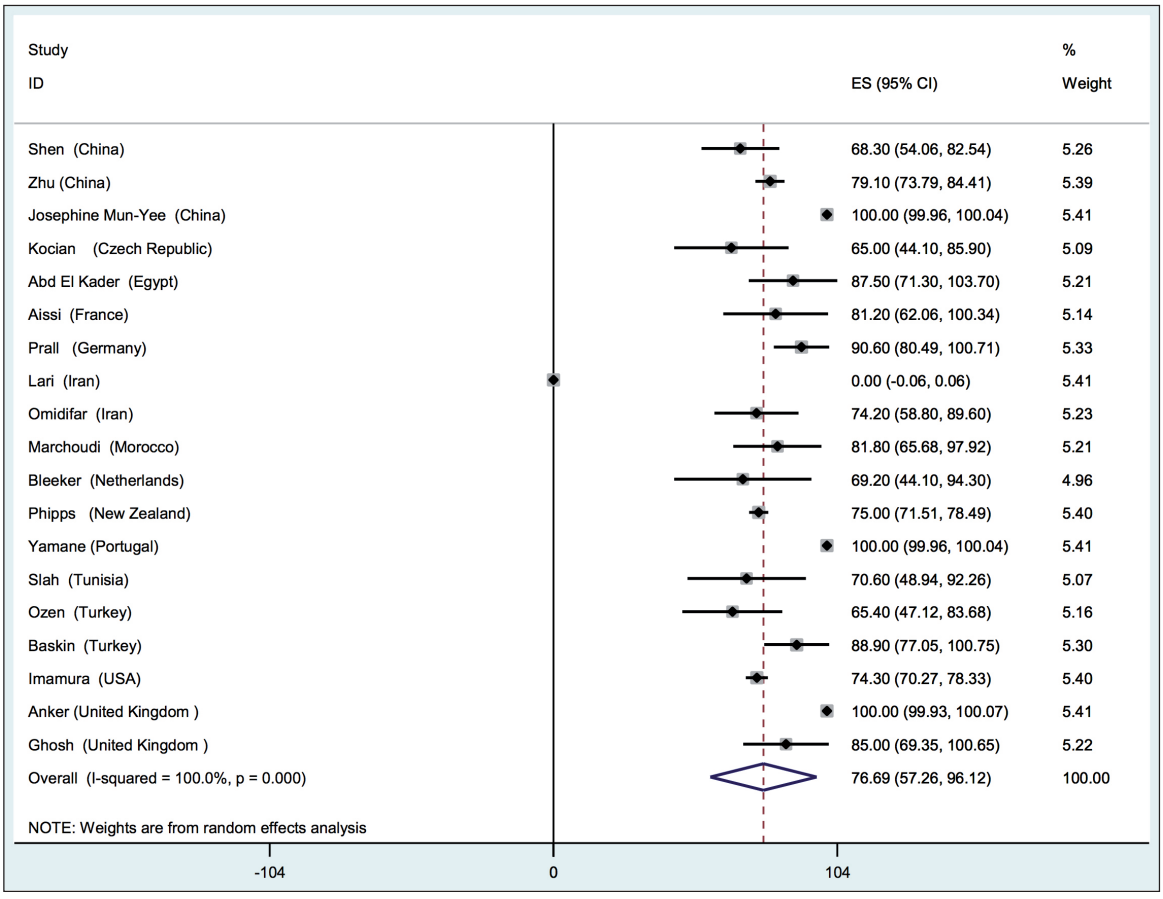

Fig. 4. Point and pooled prevalence of KRAS gene mutation in codon 12 among patients with primary colorectal cancer.

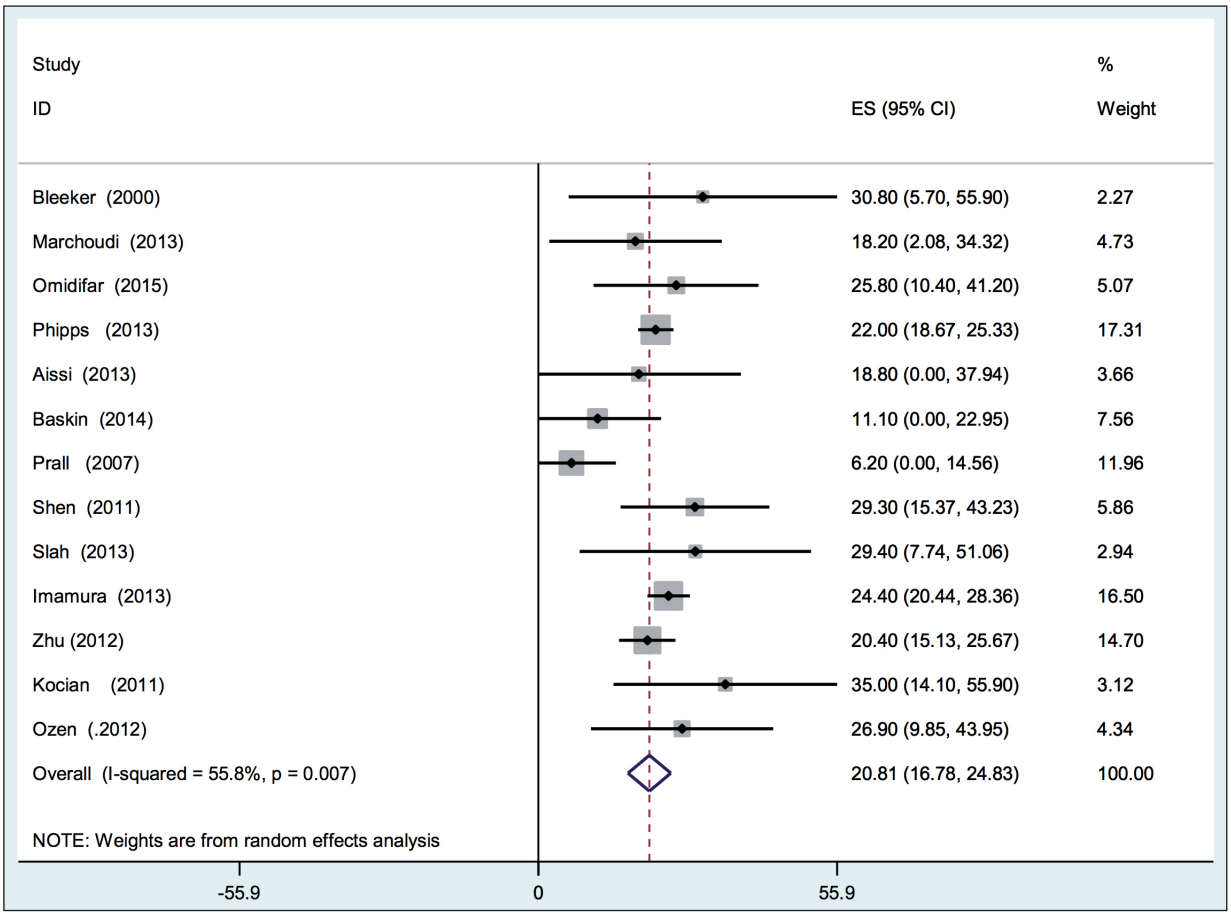

Fig. 5. Point and pooled prevalence of KRAS gene mutation in codon 13 among patients with primary colorectal cancer.

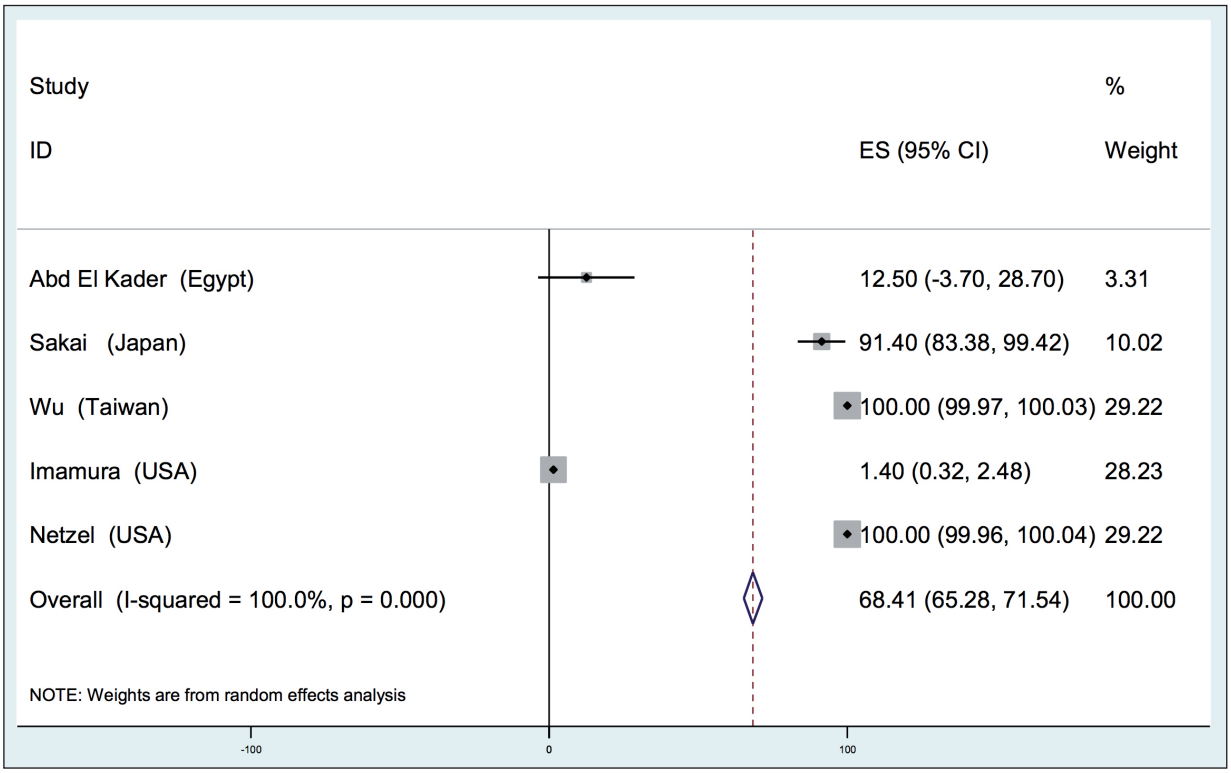

Fig. 6. Point and pooled prevalence of KRAS gene mutation in codon 12 and 13 among patients with primary colorectal cancer.

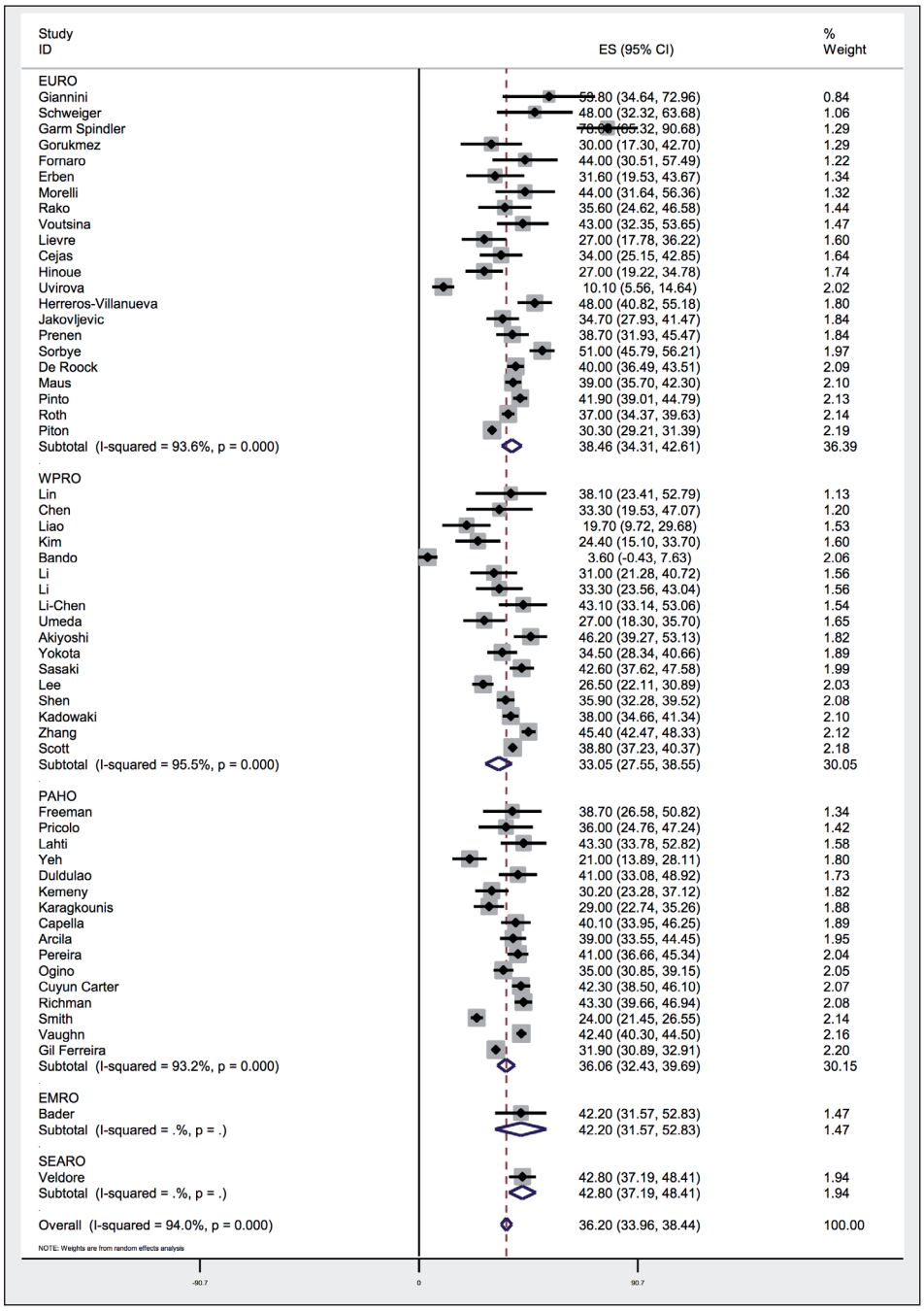

Fig. 7. Point and pooled prevalence of KRAS gene mutation among patients with metastatic colorectal cancer.

conducted in the EMRO region reported the prevalence of KRAS gene mutation among metastatic samples in Saudi Arabia as of 42.20%[43]. Another study carried out in the SEARO region by Veldore et al[26] showed the prevalence of this mutation in India as of 42.80%.

According to the results of meta-regression models, geographical region did not influence the heterogeneity of the primary results (β=-1.77, p=0.110). Moreover, funnel plot did not reveal any evidence of publication bias (Figure 8).

DISCUSSION

In the current study, mutation in KRAS gene was investigated in patients with primary and metastatic CRC. Combining the results of these primary studies, the total prevalence of KRAS gene mutation was estimated as of 33.14% in patients with primary CRC. According to different WHO regions, the prevalence of this mutation among patients with primary CRC in EMRO, EURO, PAHO, SEARO and WAPRO was 30.23%, 35.12%, 31.83%, 33.17% and

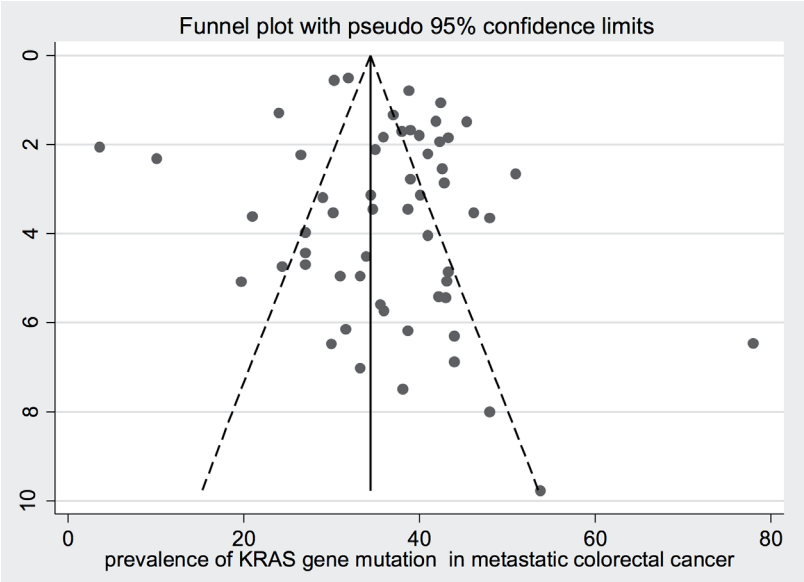

Fig. 8. Funnel plot for investigating publication bias (prevalence of KRAS gene mutation in metastatic colorectal cancer).

32.64%, respectively. Corresponding rates for mutation among metastatic cases were 42.20%, 38.46%, 36.6%, 42.80%, 33.05%, respectively. In addition, the total prevalence of KRAS gene mutation in codons 12 and 13 was estimated as of 76.69% and 28.49%, respectively.

Bishehsari et al[100] investigated the pattern of KRAS gene mutation among Iranian and Italian patients with CRC. They found that this mutation was more common among Italian patients compared to Iranian patients (46.3% *vs.* 37.4%, respectively). However, the difference was not statistically significant. Although this mutation was more common in women, it was not statistically significant. The highest mutation (66%) was observed in codon 12.

Santini et al[12] conducted another study on pathologic samples of patients with CRC aged 41-84 collected from several hospitals during 1998-2007. KRAS gene mutation was observed in 38 (38.4%) of primary tumors and 36 (36.4%) of metastatic tumors.

Dolatkhah et al[11] investigated 30 samples of patients with CRC referred to Imam Reza and Sina Hospitals in Tabriz - northwest of Iran. Of these samples, 20% had heterozygote mutation in KRAS gene and 80% had wild type. No mutation of this gene was observed in the normal tissue around tumor. Risk of mutation among patients with high grade tumor was 2.1 fold greater that that among the other patients. Presence of metastasis caused 10% increase in the chance of mutation. No significant association was observed between mutation and clinical-pathological manifestations, histological evidences, staging, tumor differentiation and familial history. Although men experienced 1.7 folds higher rates of mutation, the observed association was not statistically significant.

Cejas et al[87] investigated the specimens of 220 patients with colorectal adenocarcinoma (110 primary and 110 metastatic tumors) diagnosed during 1997-2007. KRAS mutation was observed in 34% of primary tumors and 36% of metastatic ones. No significant relationship was observed between KRAS mutation and histopathological characteristics.

Roudbari et al[9] investigated 50 samples of Iranian patients with CRC randomly selected from different parts of Iran. KRAS gene mutation was observed in 35-42% of patients. Most of which were in codon 12 and 13, respectively. No mutation was observed in codon 61. Approximately 81% of the mutations were observed among men, 24% of which were in codon 12 and 10% were in codon 13.

In another study conducted by Martinetti et al[15] among Albanian patients aged 17-85 suffering from colon cancer, frequency of KRAS gene mutation was 17.6%.

Edalat et al[10] investigated the presence of mutation in KRAS gene among 55 samples provided from Iranian patients with CRC. Of them, 36 (65%) had mutation. The rate of mutation in fresh and paraffin-embedded samples was 56% and 73%, respectively. Presence of mutation was associated just by tumor grading. A relationship was observed between mutation and poor differentiation of the tumor indicating important role of KRAS gene mutation on cell differentiation.

Shemirani et al[16] compared the KRAS gene mutations between 48 patients with CRC and 47 patients with polyp. Of cancerous patients, six mutations were observed – five in codon 12 and one in codon 13. These mutations occurred in five women and one man. Of these mutations, five were observed in colon and one occurred in rectum. Of polyp samples, two mutations occurred in codon 13 and one in codon 12.

In the study carried out by Lary et al[30] among 54 paraffin-embedded samples collected from patients with CRC from northeast of Iran, the mutation rate among patients aged under 45 was higher than that of the other age groups. It was also associated with staging of tumor, but no relationship was observed between mutation and other factors such as gender, tumor location, grade and type of cancer. The most mutations were observed in recto sigmoid.

It should be noted that mutation in KRAS gene is occurred in the initial phases of cancer development; therefore, identifying this mutation would be critical for prognosis of the patients with CRC. Early detection of cancer leads to improvement in patients' quality of life, survival and reduction in morbidity and mortality.

One of the limitations of the current study is different diagnostic methods reported in the primary studies which can play an important role in the significant heterogeneity. In addition, considerable variations in the characteristics of the study subpopulations such as genetics, environment, diet and lifestyle are another limitation of our meta-analysis. However, we combined the results using random effect model to consider such heterogeneity. Moreover, the tissue samples in the primary studies had been collected from different parts of the bowel and we had to consider them as colorectal samples.

CONCLUSIONS

Our meta-analysis showed that more than one-third of samples of patients with CRC carried KRAS gene mutation. We also found that this mutation was slightly more common in metastatic samples than primary cancers.

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Conflict of interest:
The authors declare that they have no conflict of interest.

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