Keywords
Colorectal cancer, KRAS mutations, BRAF mutations, Arab populations, Molecular profiling, Targeted therapy, Systematic review, Meta-analysis.

Authors
[1]Taghreed A Kreem, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia.

[2]Reyadh Salim Mohammed, Ibnsina University of Medical and Pharmaceutical Sciences, Iraq, Baghdad, Iraq

[3]Abbas A. Mohammed, Mustansiriyah University, Iraqi Center for Cancer and Medical Genetic Research, Department of Molecular biology, Baghdad, Iraq

[4]Ammar J. Kadhim, Ibnsina University of Medical and Pharmaceutical Sciences, Iraq, Baghdad, Iraq

[5]Shairah Abdul Razak, Department of Applied Physics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia

[6]Mohd Firdaus- Raih, Department of Applied Physics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia, Bioinformatics and Molecular Simulations Research Group, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia, Malaysia Genome and Vaccine Institute.
Email : p118269@siswa.ukm.edu.my

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Prevalence of BRAF and KRAS Mutations in Colorectal Cancer: A Systematic Review and Meta-Analysis in selected Arab Countries

Abstract

Aim The high frequency of colorectal cancer (CRC) in Arab countries indicates many people die from cancer as a result of the CRC. Both KRAS and BRAF mutations are significant factors in the acquisition of CRC, and their impact on CRC tumorigenesis and resistance to therapeutics is systemic. The aim of this research was to provide information about the frequency of KRAS and BRAF mutations in CRC patients in Arab countries (predominantly Egypt, Iraq, Saudi Arabia, and Morocco), and provide insight into the region's future molecular profile of CRC patients and how to manage treatment.

Methods After following PRISMA guidelines, we completed a systematic review and meta-analysis. We searched in PubMed, Google Scholar, and ScienceDirect for studies published from 2016 until 2024 that provided KRAS and/or BRAF mutation rates for CRC patients in Arab countries. To estimate the pooled prevalence, we utilized a random effects model. Additionally, we conducted subgroup analyses based on country, detection methods, and year of publication to explore heterogeneity in our analyses. All analyses were conducted using R version 4.4.1.

Results A total of 42 studies provided data for 7270 CRC patients for the KRAS analysis and 3361 CRC patients for the BRAF analysis.

The KRAS mutations' pooled prevalence rate was found to be 48.1% (95% CI: 42.7% to 53.6%). This high prevalence primarily occurred in codons 12 and 13 of exon 2. On the other hand, the pooled prevalence rate of BRAF mutations was initially 5.08% (95% CI: 2.53% to 9.93%), but after adjusting for significant publication bias using the trim-and-fill method, the true estimated prevalence increased to 8.5% (95% CI: 4.8% to 14.7%). The most common variant seen was V600E . In addition to identifying high prevalence for KRAS mutations, the lower prevalence of BRAF mutation was also found to be clinically significant. Additionally, the current study was characterized by significant heterogeneity related to geographic and methodological differences across included studies.

In Conclusion, This meta-analysis demonstrated that the represented Arab countries (with data heavily skewed toward Egypt, Iraq, Saudi Arabia, and Morocco) have a high prevalence of KRAS mutations and lower but still clinically relevant rate of BRAF mutations among patients with colorectal cancer. Additionally, the results demonstrate the necessity for routine molecular profiling to optimize the use of targeted therapies in Arab countries. Future studies must have broader geographic coverage and be conducted using standardized methods to enable successful implementation of precision oncology into the region's healthcare systems.

Introduction

Colorectal cancer (CRC) ranks as the third most frequently diagnosed type of cancer globally, and it continues to be one of the primary causes of cancer-related death, resulting in more than 900,000 deaths each year (1). However, CRC is becoming more prevalent in Arab nations, where it has shown a continuous increase in incidence, thereby posing a growing risk to public health. To enhance early detection, personalize therapeutic interventions, and ultimately yield better patient outcomes, it is necessary to identify the genetic drivers behind CRC, specifically, mutations that impact tumor biology and response to therapy (2).

KRAS and BRAF are two of the most important genetic alterations that take place in CRC. KRAS and BRAF mutations lead to tumor formation, progression of the disease, and resistance to targeted therapies in the case of anti-epidermal growth factor receptor (EGFR) treatments (i.e., cetuximab and panitumumab). Mutations that activate KRAS, specifically those in codons 12 and 13 in exon 2, lead to constant activation of the Ras/MAPK signaling pathway, which enhances tumor growth, thereby causing targeting of EGFR (i.e., cetuximab and panitumumab) to have minimal to no effects (3, 4). Likewise, the BRAF V600E mutation drives persistent MAPK/ERK pathway signaling and is associated with poor prognosis, aggressive tumor behavior, and a frequent association with microsatellite instability-high (MSI-H) status an important marker with therapeutic implications (5, 6).

An earlier systematic review stated that approximately 37.1% of CRC patients from Arab countries had KRAS mutations, while only 2.6% had BRAF mutations (2). However, there were no pooled meta-analytic estimates of mutation frequency, no formal evaluations of heterogeneity between studies, unmeasured publication bias, and no subtype-specific prevalence analyses were performed for those studies included by the authors. Finally, this review had fewer studies and subjects than our current meta-analyses; thus, our current study expands upon previous literature by quantitatively determining the prevalence of KRAS and BRAF mutations among Arab patients with colorectal cancer while providing country-by-country subgroup analyses and exploring sources of methodological heterogeneity.

The novelty is not broader biomarker coverage; it is the quantitative meta-analysis, larger dataset, pooled prevalence estimates, heterogeneity assessment, and mutation-level analyses

The present systematic review and meta-analysis were conducted to close these gaps by providing a summary of the pooled prevalence of KRAS and BRAF mutations found in individuals with CRC (from all Arab countries) and by also summarizing the distribution of KRAS and BRAF mutation types by exon, codon, and type of amino acid substitution. Subgroup analyses were conducted according to geographic location, timeframe for the studies included, and type of KRAS or BRAF mutation detection methods used. Analyses of variability across studies were performed and potential publication bias was assessed. Using the collected scientific data from the region provides meaningful evidence for developing molecularly-based precision oncology and thus aids in the development of strategic treatment approaches targeting Arab populations with KRAS and BRAF mutations.

Material And Methods

Study Aim, Design, and Setting

The aim of this research is to determine the combination prevalence of KRAS and BRAF gene mutations among Arab patients with colorectal cancer (CRC) and to examine differences between regions and methods used. A systematic review and meta-analysis were performed following the PRISMA guidelines (7). This study was not prospectively registered as it has a retrospective design.

Literature Search Strategy

A comprehensive literature search was performed across PubMed, Google Scholar, and ScienceDirect for studies published between 2016 and June 2024. The search strategy used combinations of keywords such as "colorectal cancer", "KRAS", "BRAF", and individual Arab country names. Detailed search terms and export formats are provided in (S File 1) (Table 1)

TABLE 1. Database Search Strategy and Export Formats for Colorectal Cancer Studies in Arab Countries

Database	Search Strategy	Export Format
Google Scholar	Advanced filters applied using keywords: "BRAF" OR "KRAS" AND "colorectal cancer" AND [Arab country names].	RefMan
PubMed	Title/Abstract search: "colorectal cancer" AND "KRAS" OR "BRAF" AND at least one Arab country in the affiliation.	Citation Manager
ScienceDirect	Title search: "BRAF/RAF" OR "KRAS/RAS" AND [Arab country affiliation].	RIS Format

Eligibility Criteria

To establish the criteria for the studies included, only studies published in English language peer-reviewed journals. Any studies performed in the 22 countries part of the League of Arab States were eligible for inclusion in this study. The countries that are part of this league include Algeria, Bahrain, the Comoros (also known as the Comoros Islands), Djibouti, Egypt, Iraq, Jordan, Kuwait, Lebanon, Libya, Mauritania, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Somalia, Sudan, Syria, the Republic of Tunisia, the United Arab Emirates, and Yemen. While all the countries that are a part of the League of Arab States were included in our search strategy, we found available studies on KRAS from only 9 of these countries, while studies available for BRAF were limited to 7 countries. Furthermore, countries without any available studies listed are reported separately; however, you should not interpret that as meaning that there is no mutation information available for those countries around the world.

○ = Searched but no eligible studies identified in PubMed, Google Scholar, or ScienceDirect (2016–June 2024). ✓ = Studies meeting inclusion criteria were identified and included.

To qualify for exclusion, abstracts, conference presentations, reviews, literature published outside of a peer-reviewed journal and those which did not have geographic information were all excluded from consideration as well as any study that did not provide pertinent information related to mutations. The remaining list of studies, after removing duplicate entries and obtaining a thorough review, totaled 42 studies that met the eligibility criteria and could be used in a meta-analysis. Titles and abstracts were independently screened by two researchers, and any disagreements were resolved through discussion (S File 2). The study selection process is illustrated in the PRISMA flow diagram (Fig 1).

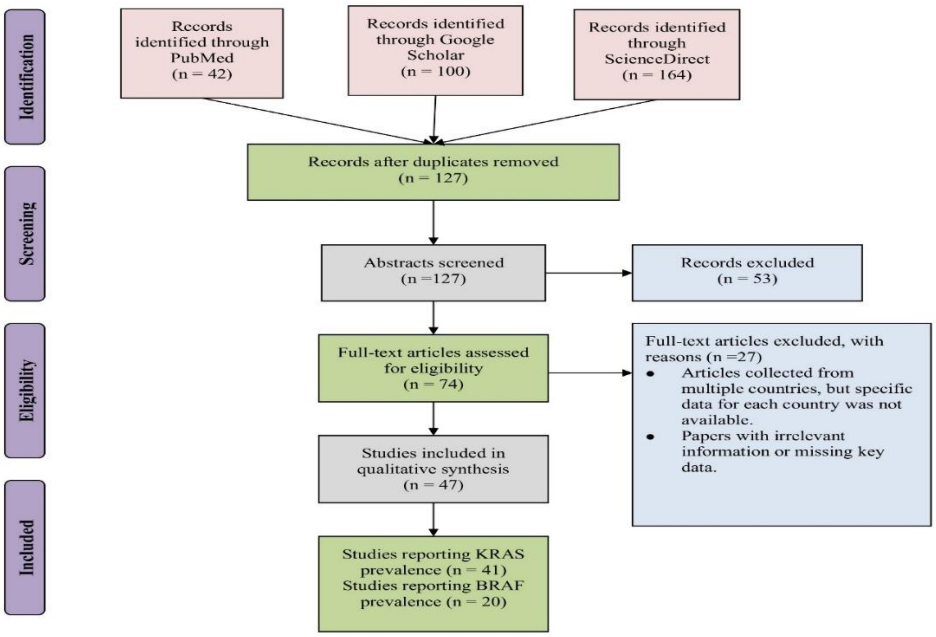

FIGURE 1. PRISMA Flow Diagram for Study Inclusion

Information source and search strategy:

A comprehensive literature search was conducted using three electronic databases:

- PubMed (MEDLINE) – searched from Jan 2016 through Jun 2024, with last update being Jun 2024.
- Google Scholar – searched from Jan 2016 through Jun 2024, with last update being Jun 2024.
- ScienceDirect – searched from Jan 2016 through Jun 2024, with last update being Jun 2024.

Trial registries, grey literature repositories, nor any other organisation's website were not searched during the process. The reference lists for all included studies and relevant systematic reviews were also screened for additional and/or potential eligible studies. Authors were not contacted in an attempt to secure any unpublished data.

The search strategies were independently devised for each of the 3 databases and each of the search strategies included combinations of a controlled vocabulary and free-text keywords. Full search strategy documentation for all 3 databases, inclusive of all filters and limits, can be found in Supplementary File 1 (and in Table 1 of the main manuscript). Specifically, the core search terms were combinations of:

- 'colorectal cancers' OR 'colon cancers' OR 'rectal cancers' OR 'colorectal carcinomas'
- 'KRAS' OR 'K-RAS' OR 'KRAS mutations' OR 'RAS mutations'
- 'BRAF' OR 'BRAF V600E' OR 'BRAF mutations'
- All of the 22 Arab League member states as geographic limits (i.e., 'Egypt' OR 'Iraq' OR 'Saudi Arabia' ...etc.).

Search was limited to publications in English only and with no further restrictions on date or filters (except for being published after the start date of 2016).

Selection Process

Records that were obtained from each of the three databases were exported, compiled into a reference management system and duplicates were removed. All titles and abstracts of records were independently screened against pre-defined inclusion and exclusion criteria by 2 independent reviewers (T.K.A and S.A.E). All articles that could not be excluded based on title/abstract alone had the full-text identified and retrieved. Full-text articles were then assessed independently by both reviewers to determine if they met final criteria for inclusion. Any disagreement at any step was resolved by discussion and consensus of both reviewers; if a consensus could not be reached, a third reviewer (S.B.M.) determined the final decision for inclusion or exclusion. The inter-reviewer agreement rate and number of disagreements resolved is reported in

Supplementary File 2. No automation tools or AI-assisted screening tools were utilized in this process. The complete study selection process can be found in the PRISMA 2020 flow diagram (Figure 1).

Data Collection Process and data items:

The data were collected by two independent reviewers (T.A.K. and S.B.M.), using a pre-defined data collection sheet (form). Any differences were resolved through discussions with each other. In the case of a study whose reporting was not clear or was incomplete, the original source of the data was reviewed in detail, and no contact with authors was made to clarify issues. There were no automated tools used to conduct the data extraction process. All data collected, were once again reviewed by the third reviewer (M.F.R.) prior to the data analysis process.

The primary outcomes were:

• Prevalence of KRAS mutations (overall; by exon 2, 3, and 4; by codon 12, 13, 59, 61, 117, 146; and by amino acid substitution).

• Prevalence of BRAF mutations (overall; by exon 11, and 15; by codon 600; and Amino Acid substitutions V600E/D, and other variants).

All results that were consistent with each of the outcomes defined above within each of the studies were reported and utilized in the extraction of data.

Study Risk of Bias Assessment

Individual studies included in the review were assessed for risk of bias. Risk assessment was based on the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Studies Reporting Prevalence Data, using an amended version containing nine items for each study that included the following:

1. Sample derived from an adequately identified population
2. Study participants recruited appropriately
3. Sufficient sample size
4. Study subjects and setting are detailed
5. Data analysis adequately represents sample
6. Identifying the condition utilized valid methods
7. Condition measured with reliability by using standard definition(s) across all subjects
8. Adequate statistical analysis
9. Response rate is adequate; if not, low response rate has been adequately addressed

Each checklist item was rated "Yes," "No," or "Unclear." Two reviewers (T.A.K. and S.A.E.) independently assessed the risk of bias for each study, with discrepancies discussed until resolved. All studies were included regardless of risk of bias as risk of bias was considered a contextual component and reported as risk of bias in sensitivity analyses.

Data Extraction and Coding

Data were independently extracted by two reviewers and included study metadata (country, year of publication, sample size, and mutation detection method) as well as genetic data (gene: KRAS or BRAF; exon, codon, amino acid alteration; and mutation frequency). Mutation detection methods were categorized as PCR-based, sequencing-based, combined PCR and sequencing, or Immunohistochemistry (IHC). KRAS mutations were grouped by exons 2, 3, and 4, while BRAF mutations were categorized under exons 11 and 15. For primary studies that reported the overall mutation rates but not the exon, codon or amino acid information, the missing variables were coded as NR (Not Reported). Methodologically, such studies were included for the overall pooled prevalence calculations but strictly excluded for the specific subgroup analyses (e.g. codon specific or exon specific prevalence) to avoid data skewing. Thus the total sample size (denominator) differs between subgroup analyses depending on availability of detailed data in the primary literature. A visual summary of the data coding scheme is provided in (S Fig1 A and B).

Statistical Analysis

Data cleaning and transformation were performed using the dplyr (8) and tidyr (9) packages in R version 4.4.1 (10). Meta-analyses were conducted using the meta and metafor packages (11), through random-effect methods was used to provide a pooled value for the estimated prevalence of mutations and the corresponding 95% confidence intervals. When studies reported a zero event (0% prevalence), a standard continuity correction of 0.5 was automatically added to all cells in the study's data table to allow calculation of variances and inclusion in the pooled meta-analysis.

The degree of heterogeneity was calculated using the I2 statistic (12), which can be interpreted to be: 25% = Little heterogeneity, 50% = Medium heterogeneity, and 75% = High heterogeneity. Forest plots were created for visualization of the detected mutation prevalence throughout all studies.

Subgroup Analyses and Visualization

Subgroup analytic methods were also done by geography (i.e., country), mutation detection method and study adjournment timeframe. There were several R packages used for visualizing data: ggplot2 (13) for general plotting, viridis and viridisLite (14) for heatmaps, and ggmap (15) for producing geographic maps with country's without data in grey.

Publication Bias Assessment

The assessment of publication bias in the meta-analysis consisted of a thorough review (visual evaluation) of funnel plots for KRAS and BRAF mutations in order to identify any potential asymmetry that may suggest publication bias (16). To determine the statistical significance of any identified asymmetry, a formal test of the asymmetry was performed using Egger's regression test, and a two-sided p-value < 0.05 was defined as significant. In addition, the trim-and-fill procedure was used to calculate the estimated number of missing studies and to obtain adjusted prevalence estimates (17). As well as interpreting all results based upon the statistical significance, a consideration of the clinical significance and implications for the management of colorectal cancer among Arab populations was also undertaken. This extensive evaluation increased the reliability and comprehensiveness of the results of the meta-analysis relating to the prevalence of KRAS and BRAF mutations.

TABLE 2. here inserted

Table: 2 Classification of all 22 Arab League member states by study availability status in KRAS and BRAF analyses

Arab League Member State	KRAS Analysis Status	BRAF Analysis Status
Algeria	✓ Included	✓ Included
Bahrain	○ Searched, no studies	○ Searched, no studies
Comoros	○ Searched, no studies	○ Searched, no studies
Djibouti	○ Searched, no studies	○ Searched, no studies
Egypt	✓ Included	✓ Included
Iraq	✓ Included	✓ Included
Jordan	✓ Included	○ Searched, no studies
Kuwait	○ Searched, no studies	○ Searched, no studies
Lebanon	✓ Included	✓ Included
Libya	✓ Included	○ Searched, no studies
Mauritania	○ Searched, no studies	○ Searched, no studies
Morocco	✓ Included	✓ Included
Oman	○ Searched, no studies	○ Searched, no studies
Palestine	○ Searched, no studies	○ Searched, no studies
Qatar	○ Searched, no studies	○ Searched, no studies
Saudi Arabia	✓ Included	✓ Included
Somalia	○ Searched, no studies	○ Searched, no studies
Sudan	○ Searched, no studies	○ Searched, no studies
Syria	○ Searched, no studies	○ Searched, no studies
Tunisia	✓ Included	✓ Included
United Arab Emirates	○ Searched, no studies	○ Searched, no studies
Yemen	○ Searched, no studies	○ Searched, no studies

1. NR = Not Reported (variable not specified in original study)
2. Imm.histo. = Immunohistochemistry
3. KRAS and BRAF sample sizes differ, so listed as "KRAS / BRAF"
4. Used only if two studies share the same first author and year

Risk of Bias Evaluation:
The JBI Critical Appraisal Checklist was used to assess the overall quality of the included studies and was considered acceptable for meta-analysis. Most of the studies had a low to moderate risk of bias with high scores mainly in the identification of the sample and valid measurement of the condition. However, some studies were downgraded due to the lack of clear reporting of recruitment strategies and failure to address confounders. However, these variations did not result in any studies being excluded entirely based on risk of bias, and this methodological heterogeneity contributes to the overall heterogeneity observed

Results
Study Characteristics:
This meta-analysis included a total of 42 eligible studies, comprising 36 studies on KRAS mutations and 18 studies on BRAF mutations, conducted across 9 and 7 Arab countries, respectively (Table 2; S Files 3 and 4). The pooled sample size was 7,270 patients for KRAS analysis and 3,361 patients for BRAF. The included studies were published between 2016 and June 2024 and primarily

employed PCR-based methods, sequencing techniques, Immunohistochemistry, or combinations of these approaches for mutation detection.

Prevalence of KRAS Mutations in CRC Patients:

The pooled prevalence of KRAS mutations among colorectal cancer (CRC) patients across Arab countries was 48.10% (95% CI: 42.7–53.6%), with substantial between-study heterogeneity (I^2 = 89.3%, p < 0.0001) (Fig 2). Prevalence estimates varied widely across studies, ranging from 0% (18) to 87.5% (Alsolme et al., 2023) [28](19). Exon 2 mutations were the most frequently reported, with a pooled prevalence of 41.89%, underscoring their clinical importance in CRC pathogenesis (Table 2, S File 5). In contrast, exon 3 and exon 4 mutations were considerably less common, with pooled prevalence rates of 2.01% and 4.10%, respectively. At the codon level, the most frequently mutated codon was codon 12 (34.73%), followed by codon 13 (7.17%). Both CODON 12 and CODON 13 had high levels of mutation variability within and among the studies. The relative frequency of mutations at the rest of the codons was low.

At the amino acid level, the most prevalent mutations, including the number of different types of alteration, were the following: p.G12D (13.80%), p.G12V (9.78%), and p.G13D (6.76%). Four additional miscellaneous amino acid variants were identified with a combined prevalence of the p.A146P/T/V variants (3.86%), and most of them had relatively low levels of variation (Table 3, supplied file 5). There were also geographical differences in the pattern of mutations (Figure 3). For instance, in Egypt, the prevalence for the p.G12D mutation was 21.05% (highest), in Iraq, the prevalence for p.G13D mutation was 35.96% (highest) and p.G12C mutation was 27.09% (highest), and the highest prevalence of the p. G12V mutation was found in Libya (15.62%) and Tunisia (14.29%). The geographical differences may reflect population differences in inherited genes, environmental toxins, or methodologies used in the diagnosis of mutations. Due to the extremely high heterogeneity (I^2 = 89.3%), pooled estimates should be interpreted with caution. A nonparametric summary was conducted in conjunction with the random-effects model, and a median KRAS mutation prevalence was 46.5% (interquartile range [IQR]: 39.0% - 53.6%), which provides a good representation of the centre of tendency, despite the dispersion of data.

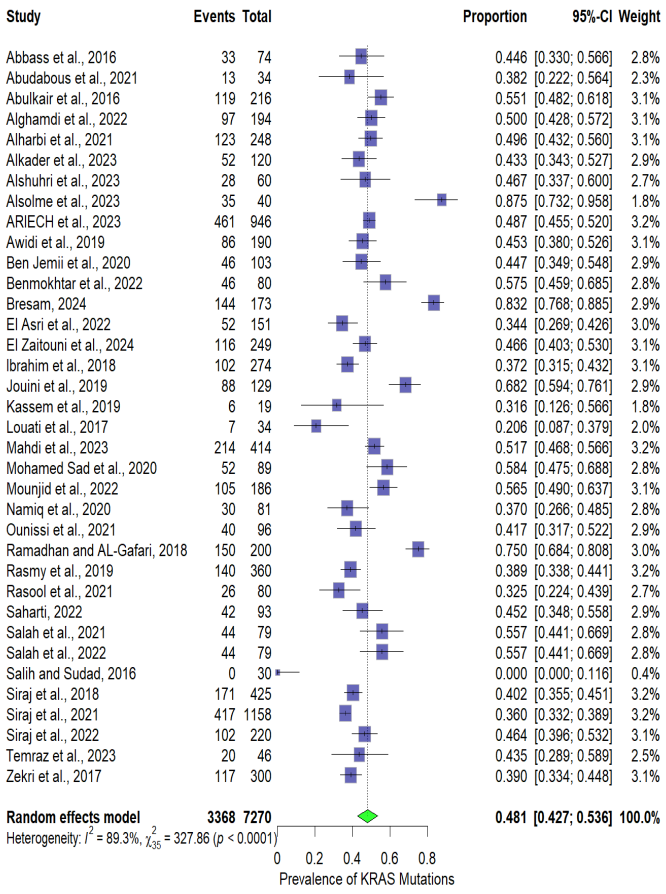

FIGURE 2. Forest plot for the prevalence of KRAS mutation in Studies done in the Arabic CRC patients

TABLE 3. Meta-analysis results for KRAS Mutation by Exons, codons and amino acid alterations

	N	S	M	P (%)	95%-CI	Tau	Tau²	I²	H	Q	d.f.	P-value
EXONS	Meta Analysis by Exons											
E2	22	3830	1588	41.89%	[35.65%; 48.41%]	0.4768	0.2273	87.0%	2.77	161.01	21	< 0.0001
E3	13	2913	58	2.01%	[1.41%; 2.87%]	0.3097	0.0959	18.2%	1.11	14.67	12	0.2600
E4	12	2839	112	4.10%	[3.35%; 5.02%]	0.0782	0.0061	0.0%	1.00	10.13	11	0.5188
E2-3-4 (mix)	8	2113	220	4.44%	[1.15%; 15.60%]	1.4909	2.2227	97.1%	5.91	244.85	7	< 0.0001
Codons	Meta Analysis by Codons											
C12	23	3870	1297	34.73%	[30.06%; 39.71%]	0.3715	0.1380	79.7%	2.22	108.32	22	< 0.0001
C13	23	3870	319	7.17%	[5.26%; 9.69%]	0.6712	0.4505	89.6%	3.10	211.49	22	< 0.0001
C59	10	2666	9	0.57%	[0.35%; 0.91%]	0.0000	0.0000	0.0%	1.00	5.01	9	0.8337
C61	13	2913	49	1.87%	[1.30%; 2.69%]	0.2287	0.0523	11.0%	1.06	13.48	12	0.3352
C117	11	2759	16	0.91%	[0.51%; 1.61%]	0.4038	0.1631	11.4%	1.06	11.29	10	0.3352
C146	12	2839	96	3.61%	[2.82%; 4.60%]	0.1003	0.0101	8.8%	1.05	12.06	11	0.3590
Mix	8	2113	220	4.44%	[1.15%; 15.60%]	1.4909	2.2227	97.1%	5.91	244.85	7	< 0.0001
AA	Meta Analysis by Amino Acid Alterations											
p. G12D	20	3123	396	13.80%	[11.52%; 16.45%]	0.2803	0.0785	54.8%	1.49	42.04	19	0.0018
p. G12V	19	3104	318	9.78%	[7.53%; 12.61%]	0.4608	0.2124	68.9%	1.79	57.96	18	< 0.0001
p. G13D	21	3296	273	6.76%	[4.74%; 9.55%]	0.7364	0.5423	90.4%	3.23	208.36	20	< 0.0001
Other. Mix	8	2113	220	4.44%	[1.15%; 15.60%]	1.4909	2.2227	97.1%	5.91	244.85	7	< 0.0001
p. G12C	19	3222	196	5.29%	[3.39%; 8.16%]	0.8332	0.6943	89.6%	3.10	173.13	18	< 0.0001
p. A146P/T/V	11	2565	92	3.86%	[3.13%; 4.74%]	0.0020	<0.0001	0.0%	1.00	8.32	10	0.5976
p. G12S	17	2950	79	3.26%	[2.10%; 5.04%]	0.7324	0.5364	75.3%	2.01	64.89	16	< 0.0001
p. G12A	16	2956	82	2.92%	[2.09%; 4.06%]	0.4658	0.2170	49.0%	1.40	29.42	15	0.0142
p. G12R/T/I/L	15	2765	52	2.14%	[1.37%; 3.32%]	0.5841	0.3412	53.6%	1.47	30.19	14	0.0072
p. Q61L/R/H/E	11	2565	46	2.03%	[1.39%; 2.97%]	0.1633	0.0267	14.2%	1.08	11.65	10	0.3090
p. G13C/A/S/V/R	7	806	8	1.49%	[0.61%; 3.62%]	0.5408	0.2924	6.9%	1.04	6.44	6	0.3754
p.K117N/E_I11A	9	2071	16	1.05%	[0.61%; 1.79%]	0.3586	0.1286	0.0%	1.00	7.65	8	0.4686
p. A59T/E/G/V	9	2392	9	0.60%	[0.36%; 0.97%]	0.0000	0.0000	0.0%	1.00	4.33	8	0.8263

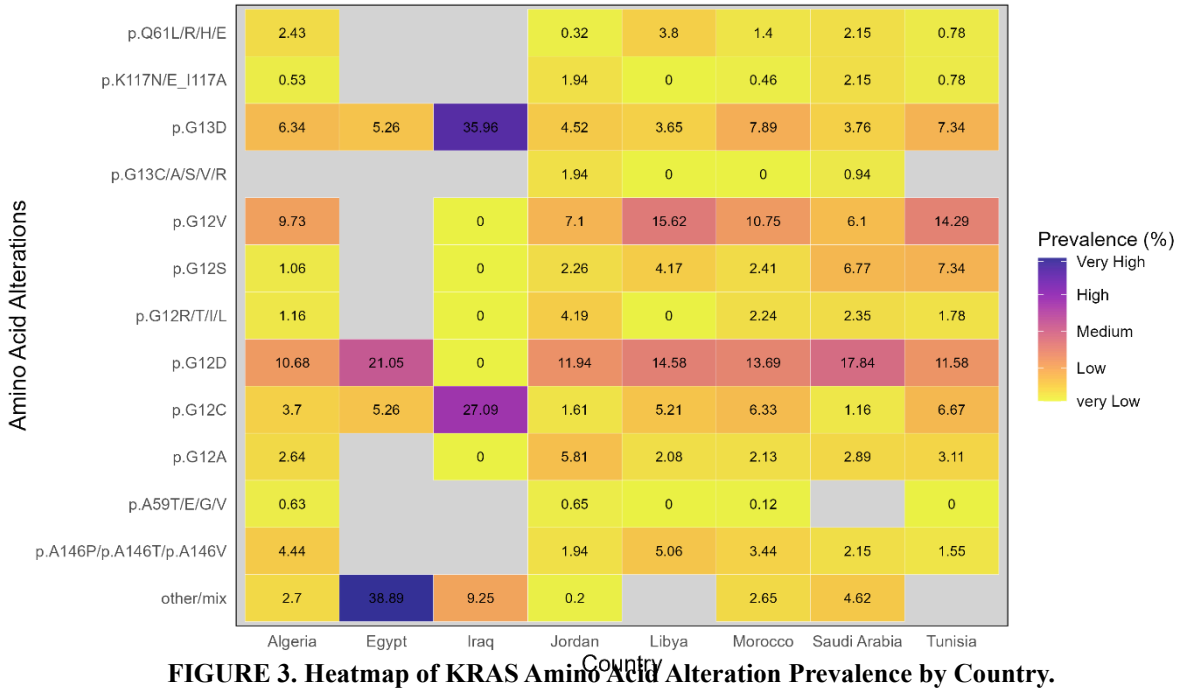

FIGURE 3. Heatmap of KRAS Amino Acid Alteration Prevalence by Country.

Subgroup Analysis of KRAS Mutations by Study Location, Period, and Detection Method

Subgroup analyses were conducted to explore potential sources of heterogeneity in KRAS mutation prevalence. The results are shown in (Table 4) and (S File 6) unless otherwise indicated. There was a statistically significant geographic variation (Q = 17.92, p = 0.0218) (Figure 4). The highest pooled prevalence was found in Libya at 52.07% (95% confidence interval: 31.25% to 72.20%), while the lowest pooled prevalence was in Lebanon at 38.14% (95% CI: 15.92 to 66.75%). Of the countries that had more than one study, Iraq had the greatest level of heterogeneity (I^2 = 95.3%) followed by Tunisia (I^2 = 90.1%) and Egypt (I^2 = 83.0%). Additional data were reported in Jordan (44.52%), Algeria (48.73%), Morocco (48.50%), and Saudi Arabia (46.56%). Temporal analyses indicate that there was a higher pooled prevalence (49.96%, 95% CI: 43.24% to 56.69%) for studies published after 2020 than for those published prior to 2020 (45.04%, 95% CI: 35.21% to 55.27%), but the difference was not statistically significant (Q = 0.73, p = 0.3931), and there was still a high amount of heterogeneity in both time frames (I^2 > 87%).

To analyze the study results further, we created sub-groups based on the mutation detection method used. By looking at the IHC (Immunohistochemistry) studies alone, we found the highest prevalence (n=1) of KRAS mutations, at 58.43% (95% CI: 47.97%–68.18%). The highest pooled prevalence (n=10) across a number of studies was seen when using sequencing techniques (i.e., genetic sequencing) at 53.57% (95% CI: 37.90%–68.56%), while PCR (Polymerase Chain Reaction) tests had the second highest pooled prevalence at 48.55% (95% CI: 42.46%–54.69%). The fewest incidences of KRAS mutation were found in PCR studies using combined methods at 41.75% (95% CI: 25.97%–59.42%). When no information was available concerning the specific detection methodologies, the overall pooled prevalence of KRAS mutations was 46.51% (95% CI: 0.82%–98.92%), which was not statistically different (Q = 4.40, p = 0.3551; df = 4) from the findings among any of the five different groups analyzed above. On the whole, the overall pooled prevalence of KRAS mutations was calculated to be 48.10%, but this figure represents a high degree of variation among the different studies (I^2 = 89.3%). Although subgroup analyses have been helpful in developing hypotheses regarding potential reasons for variability (e.g., by location, year of study, and type of detection methods used), the high degree of variability among studies suggests that caution should be used in interpreting these findings. Additionally, in conjunction with the pooled estimates, the median KRAS mutation frequency across all 10 studies was found to be 46.5% (IQR = 39.0% to 53.6%), which may be a more consistent measure of central tendency than the pooled result.

TABLE 4. Subgroup analysis of KRAS mutations prevalence of CRC patients stratified by location, period and method of study

Subgroup	N	P (%)	95%-CI	tau^2	tau	Q	I^2 (%)	PValue	DF
Country	Subgroup by Country								
Algeria	1	48.73	[45.56%; 51.92%]	--	--	0.00	--	NA	Inf
Egypt	3	44.26	[16.77%; 75.79%]	0.2331	0.4828	11.75	83.0%	0.0028	2
Iraq	4	45.45	[1.86%; 97.34%]	4.4554	2.1108	64.09	95.3%	< 0.0001	3
Jordan	2	44.52	[33.10%; 56.55%]	0	0	0.11	0.0%	0.7391	1
Lebanon	2	38.14	[15.92%; 66.75%]	0	0	0.65	0.0%	0.4200	1
Libya	3	52.07	[31.25%; 72.20%]	0.0276	0.1663	3.34	40.1%	0.1881	2
Morocco	6	48.50	[39.56%; 57.54%]	0.0896	0.2994	21.17	76.4%	0.0008	5
Saudi Arabia	11	46.56	[37.39%; 55.97%]	0.1780	0.4219	72.41	86.2%	< 0.0001	10
Tunisia	4	44.20	[17.17%; 75.17%]	0.6105	0.7813	30.25	90.1%	0.0001	3
Between Groups	-	-	-	-	-	17.92	-	0.0218	8
Period	Subgroup by period								
Before 2020	15	45.04%	[35.21%; 55.27%]	0.3362	0.5798	142.70	90.2%	< 0.0001	14
After 2020	21	49.96%	[43.24%; 56.69%]	0.2677	0.5174	184.75	89.2%	< 0.0001	20
Between Groups	-	-	-	-	-	0.73	-	0.3931	1
Method	Subgroup by Method								
Both PCR and Sequencing	8	41.75%	[25.97%; 59.42%]	0.6695	0.8182	119.03	94.1%	< 0.0001	7
Immunohistochemistry	1	58.43%	[47.97%; 68.18%]	--	--	0.00	--	NA	Inf
NR	2	46.51%	[0.82%; 98.92%]	0.2343	0.4841	7.54	86.7%	0.0060	1
PCR-based	17	48.55%	[42.46%; 54.69%]	0.1277	0.3574	97.60	83.6%	< 0.0001	16
Sequencing	8	53.57%	[37.90%; 68.56%]	0.4184	0.6469	53.56	86.9%	0.0000029	7
Between Groups	-	-	-	-	-	4.40	-	0.3551	4

N = no of studies, P = prevalence, CI = Confidence Interval, Tau = Between-study standard deviation, Tau² = Between-study variance, I² = Percentage of total variation due to heterogeneity, H = Heterogeneity statistic, Q = Cochran's Q statistic, df = Degrees of Freedom, NR=non reported

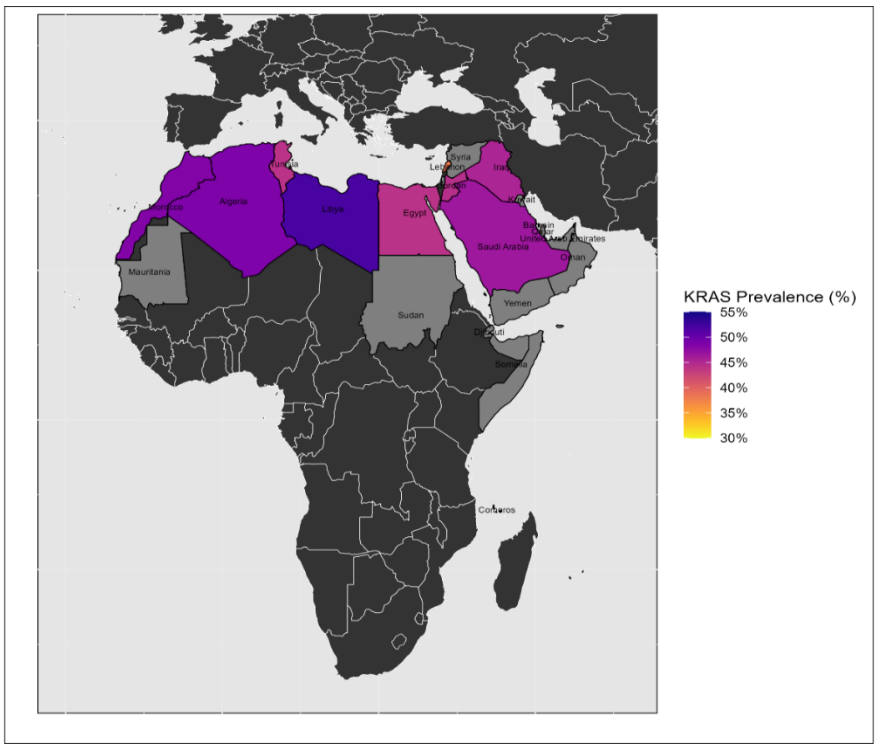

FIGURE 4. Geographic Distribution of KRAS Mutation Rates Across Arab Countries.

Prevalence of BRAF Mutations in CRC Patients

The pooled prevalence of BRAF mutations among colorectal cancer (CRC) patients across all included studies was 5.08% (95% CI: 2.53–9.93%), with substantial heterogeneity (I^2 = 91.2%, p < 0.0001) (Fig 5). Prevalence estimates varied widely. The highest reported prevalence was 51.85% (20), while several studies reported 0% prevalence, including those by Abbood and Aziz (21) , El Asri, Ouldim (22),Mahdi, Khmou (23), Masri, Al Housseiny (24), and Ounissi, Weslati (25).

The distribution of BRAF mutations by exon, codon, and amino acid alteration is detailed in (Table 5 and S File 7), Exon 15 was the most mutated region, with a pooled prevalence of 6.18% (95% CI: 2.72–13.43%) across 14 studies, accompanied by high heterogeneity (I^2 = 93.4%, p < 0.0001). Exon 11 mutations were rare, reported in only two studies, with a pooled prevalence of 0.40%. At the codon level, codon 600 was the most frequently mutated, showing a pooled prevalence of 4.72% (95% CI: 1.78–11.92%) across 12 studies, again with significant heterogeneity (I^2 = 92.7%, p < 0.0001).

The most prevalent amino acid alterations were p.V600E/D, with a pooled prevalence of 5.34% (95% CI: 1.97–13.70%) across 11 studies and similarly high heterogeneity (I^2 = 93.1%, p < 0.0001).

As illustrated in Figure 6, the geographic location all forms of BRAF mutations: The highest percentage of pV600E/D mutations occurred in Egypt (51.85%), and none reported for Tunisia (0%). Non-V600E mutations were most frequently observed in Iraq (13.33%).

These findings highlight the overall low prevalence but high variability of BRAF mutations in CRC among Arab populations, with potential implications for targeted therapy decisions and regional biomarker screening strategies.

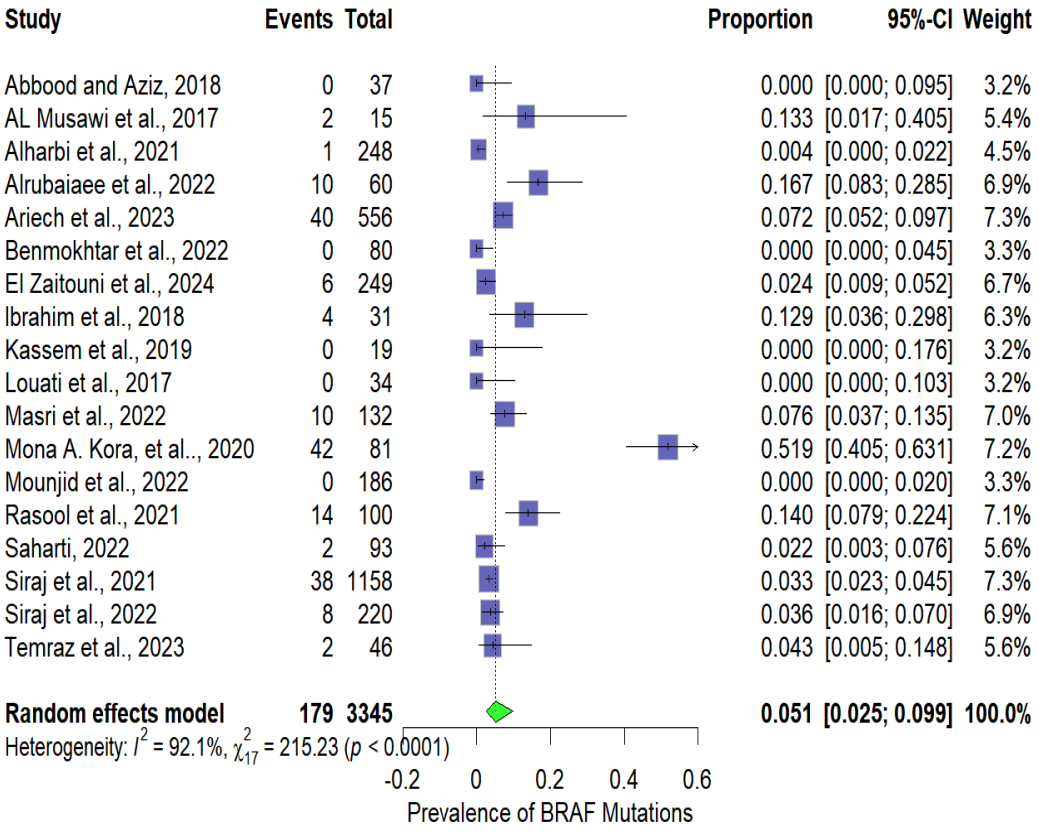

FIGURE 5. Forest plot for the prevalence of BRAF mutation in Studies done in the Arabic CRC patients

TABLE 5. Meta analysis results of the Prevalence of BRAF Mutations by Codon and Amino Acids alterations in CRC Patients

	N	S	M	P (%)	95%-CI	tau^2	tau	I^2	H	Q	df	p-value
Exons	Meta analysis of Exons											
EXON_15	14	2812	166	6.18%	[2.72%; 13.43%]	1.6166	1.2715	93.4%	3.91	198.46	13	<0.0001
EXON_11	2	636	2	0.40%	[0.03%; 5.95%]	0	0	0.0%	1.00	0.12	1	0.7322
Codons	Meta analysis of Codons											
Codon 600	12	1623	112	4.72%	[1.78%; 11.92%]	1.9568	1.3988	92.7%	3.70	150.51	11	<0.0001
other Codons	6	1940	56	4.09%	[0.92%; 16.39%]	1.6031	1.2661	83.6%	2.47	30.42	5	0.0001
	Meta analysis of amino acid alterations (AA)											
p.V600E_D	11	1543	112	5.34%	[1.97%; 13.70%]	1.8712	1.3679	93.1%	3.82	145.98	10	<0.0001
Other Mutations	5	1860	56	4.93%	[0.93%; 22.34%]	1.5745	1.2548	86.0%	2.68	28.64	4	<0.0001

N= no of studies, S = total sample size, M= total mutations, P= prevalence, CI = Confidence Interval, Tau = Between-study standard deviation, Tau² = Between-study variance, I² = Percentage of total variation due to heterogeneity, H = Heterogeneity statistic, Q = Cochran's Q statistic, df= Degrees of Freedom, AA=Amino acid alteration

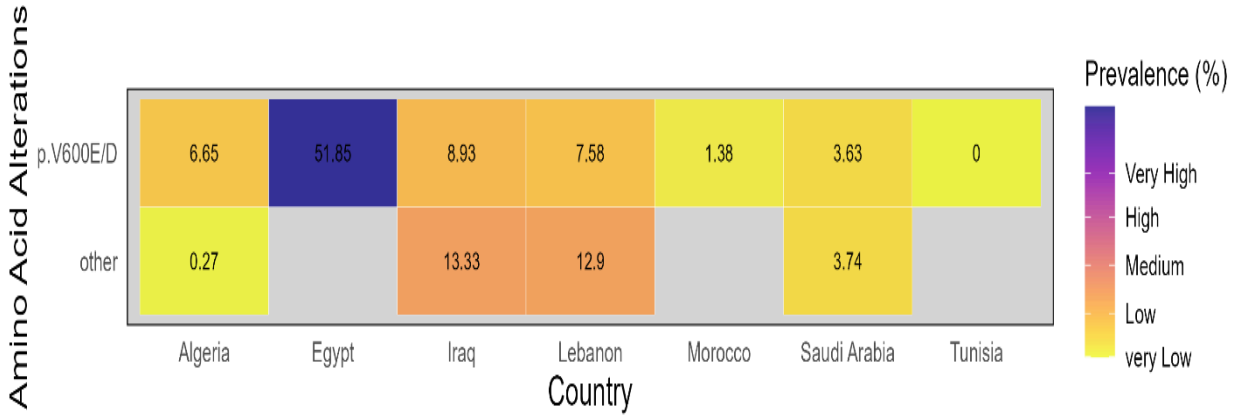

FIGURE 6: Heatmap of BRAF Amino Acid Alteration Prevalence by Country.

Subgroup Analysis of BRAF Mutations by Study Location, Period, and Detection Method

Subgroup meta-analysis demonstrated statistically significant geographic heterogeneity in BRAF mutation prevalence among CRC patients across Arab countries (Q = 15.82, p = 0.0148), (Table 6 and S File 8). The highest pooled number of cases of BRAF mutations was reported from Egypt with a prevalence of 17.84% (95% CI 0.00-100.00%) and the second highest from Iraq with a prevalence of 14.44% (95% CI 2.89-48.90%).Other countries exhibited lower prevalence estimates: Lebanon: 8.03% (95% CI: 2.90%–20.37%), Algeria: 7.19% (95% CI: 5.32%–9.66%), Saudi Arabia: 3.41% (95% CI: 0.77%–13.83%), Tunisia: 1.43% (95% CI: 0.09%–19.12%), Morocco: 1.21% (95% CI: 0.08%–16.60%).

Geographic Location Might Effect BRAF Mutation Rate The geographical location of a study could have a major impact on how often BRAF mutations occur. This may be caused by genetic, environmental, or methodical factors that differ between places. There was no statistical difference in the occurrence of BRAF mutations among the studies published before 2020 versus studies published after 2020 (p = 0.2687). The percentage of studies published after 2020 indicated a lower pooled prevalence of BRAF mutations at 4.15% as compared to 9.42% for studies published prior to 2020. However, both published groups exhibited high variability ($I^2 > 80\%$) so drawing conclusions from these findings is difficult. There was a statistically significant difference (p = 0.0050) in the occurrence of BRAF mutations observed in the studies stratified by method used for detecting mutations.

The highest prevalence of BRAF mutations, in the one study reporting the use of Immunohistochemistry (IHC) was 32.15% (95% Confidence Interval [CI]: 0.00%–100%) based solely on this one publication. The studies that used sequencing methods to detect BRAF mutations reported an overall pooled prevalence of BRAF mutations of 3.02% (95% CI: 0.85%–10.12%). Studies that utilized PCR methods produced a pooled prevalence of 2.23% (95% CI: 0.41%–11.18%) with moderate variability. Studies that combined the use of both PCR and sequencing methods produced an overall pooled prevalence of 6.80% (95% CI: 3.44%–13.02%).

Due to the high level of heterogeneity noted in the summary analysis for BRAF mutation analyses ($I^2 = 91.2\%$), caution should be used in the interpretation of pooled estimates. Therefore, to calculate a more robust estimate of central tendency, a non-parametric summary was produced for the BRAF mutation prevalence across all studies. The estimated median prevalence of BRAF mutations across all studies was 4.1% with an interquartile range (IQR) between 2.0% and 7.2%. It is anticipated that using these distributional statistics to establish a benchmark will be useful in settings where molecular testing has been limited and/or where the quality of data has been inconsistent.

TABLE 6. Prevalence of BRAF Mutations by Country, Period, and Detection Method in Subgroup Analyses

Subgroup	N	P (%)	95%-CI	tau^2	tau	Q	I^2 (%)	P-value	DF
Country	Subgroup by Country								
Algeria	1	7.19%	[5.32%; 9.66%]	--	--	0.00	--	NA	Inf
Egypt	2	17.84%	[0.00%; 100.00%]	5.9347	2.4361	6.65	85.0%	0.0099	1
Iraq	3	14.44%	[2.89%; 48.90%]	0.0001	0.0027	3.43	41.8%	0.1796	2
Lebanon	3	8.03%	[2.90%; 20.37%]	0.0001	0.0002	1.83	0.0%	0.4013	2
Morocco	3	1.21%	[0.08%; 16.60%]	0.6841	0.8271	2.94	31.9%	0.2304	2
Saudi Arabia	5	3.41%	[0.77%; 13.83%]	1.0675	1.0332	29.86	86.6%	<0.0001	4
Tunisia	1	1.43%	[0.09%; 19.12%]	--	--	0.00	--	NA	Inf
Between Groups	-	-	-	-	-	15.82	-	0.0148	6
Period	Subgroup by Period								
Before 2020	6	9.42%	[1.62%; 39.61%]	2.3694	1.5393	35.76	86.0%	<0.0001	5
After 2020	12	4.15%	[2.01%; 8.37%]	0.8344	0.9135	62.28	82.3%	<0.0001	11
Between Groups	-	-	-	-	-	1.22	-	0.2687	1
Method	Subgroup by Method								
Both PCR and Sequencing	8	6.80%	[3.44%; 13.02%]	0.4058	0.6370	33.09	78.8%	<0.0001	7
Immunohistochemistry	2	32.15%	[0.00%; 100.00%]	1.3324	1.1543	16.73	94.0%	<0.0001	1
PCR-based	5	2.23%	[0.41%; 11.18%]	1.1668	1.0802	15.01	73.4%	0.0046815	4
Sequencing	3	3.02%	[0.85%; 10.12%]	0.0000	0.0000	1.81	0.0%	0.4038088	2
Between Groups	-	-	-	-	-	12.84	-	0.0050	3

N = no of studies, P = prevalence, CI = Confidence Interval, Tau = Between-study standard deviation, Tau² = Between-study variance, I² = Percentage of total variation due to heterogeneity, H = Heterogeneity statistic, Q = Cochran's Q statistic, df= Degrees of Freedom

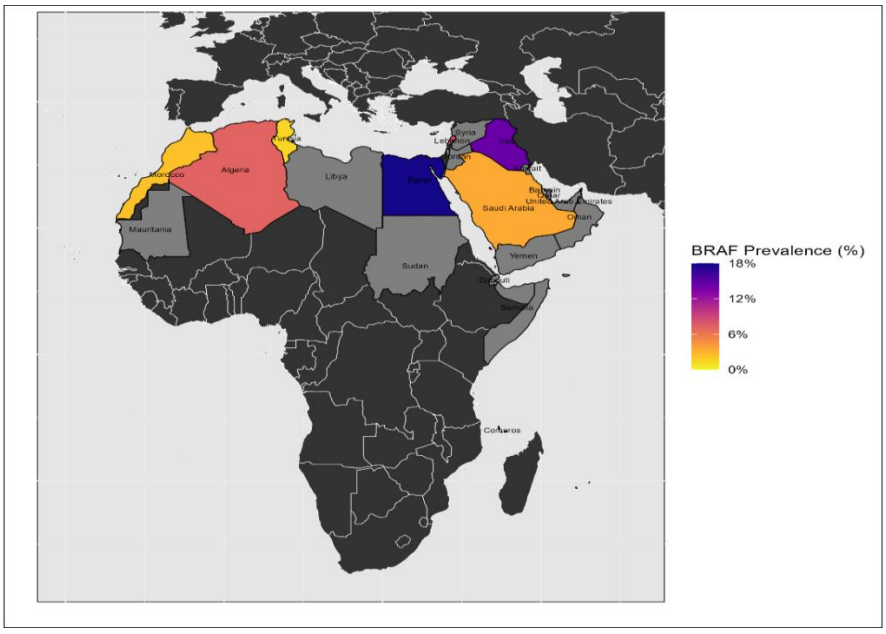

FIGURE 7. Geographic Distribution of BRAF Mutation Rates Across Arab Countries.

Examination of Publication Bias

Egger's linear regression statistical analysis has been performed to evaluate the potential for a bias in the research that has been conducted in connection with the KRAS and BRAF mutation studies. Based on the results from Egger's funnel plot asymmetry (p=0.2191) for KRAS mutation studies and (p=0.3512) for BRAF mutation studies, there does not appear to be significant evidence of any publication bias in these studies, thereby supporting the reliability and consistency of the pooled prevalence estimates for both KRAS and BRAF mutations. The trim and fill method of statistical analysis was utilized to assess the effects and likelihood of a bias and subsequently to adjust the overall pooled prevalence estimates if necessary. Where KRAS mutations are concerned, the trim and fill method did not impute any additional studies into the pooled prevalence results, and thus the adjusted pooled prevalence remains at 48.1% (95% CI: 42.7%-53.6%), thereby confirming that the original estimate is robust and not affected by any publication bias. With regard to BRAF mutations, however, a total of seven imputed potentially missing studies provided a revised adjusted pooled prevalence estimate of 8.5% (9 bias associat fore addition ular epidem for publica g of the grea and more ex

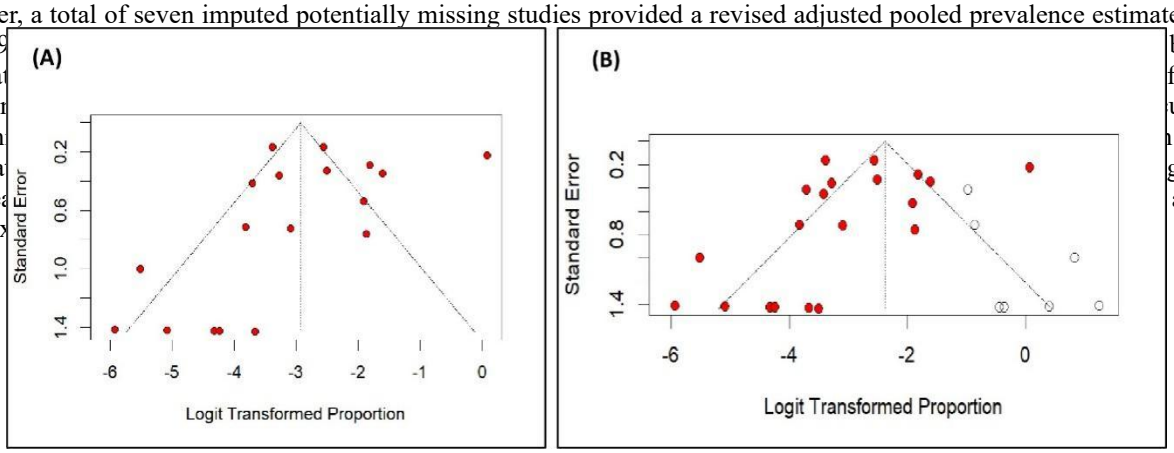

FIGURE 8. Funnel plots of KRAS mutation prevalence in CRC patients from Arab countries. (A) Observed studies. X axis: logit-transformed proportion (i.e., prevalence on the logit scale); Y axis: standard error of the logit proportion. Egger's regression test: t = 1.25, df = 34, p = 0.2191; bias estimate = 1.3442 (SE = 1.0736). (B) Trim-and-fill adjustment: no studies imputed; adjusted pooled prevalence remained unchanged at 48.10% (95% CI: 42.70–53.60%).

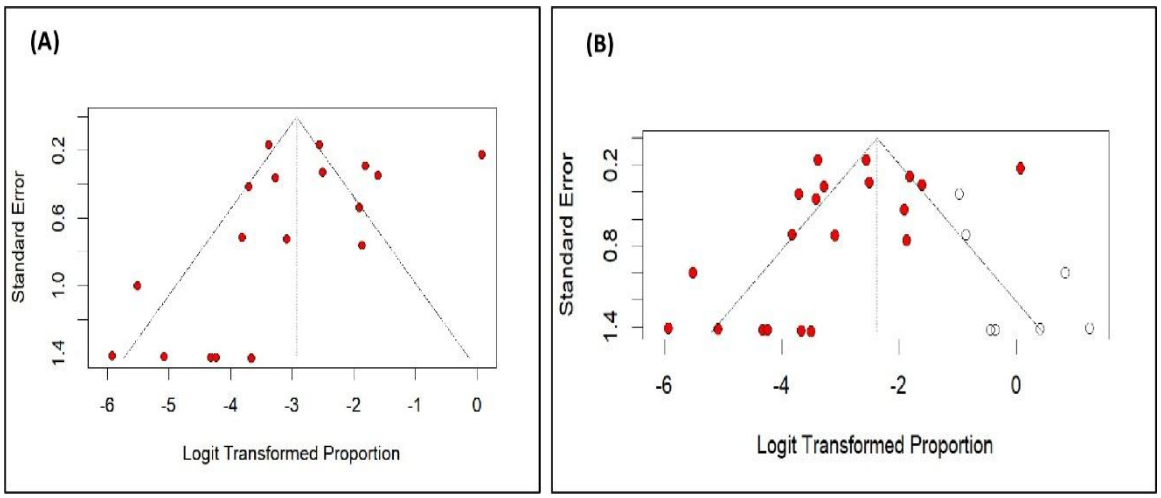

FIGURE 9. Funnel plots of BRAF mutation prevalence in CRC patients from Arab countries. (A) Observed studies. X axis: logit-transformed proportion; Y axis: standard error of the logit proportion. Egger's regression test: t = –0.96, df = 16, p = 0.3512; bias estimate = –1.3558 (SE = 1.4117). (B) Trim-and-fill adjustment: seven studies imputed; adjusted pooled prevalence increased to 8.5% (95% CI: 4.80–14.70%).

Discussion:

The meta-analysis presents an assessment of the incidence of KRAS and BRAF mutations among CRC patients within the Arab world, and how they are distributed throughout the region. The pooled incidence of KRAS mutations was observed to be 48.10%, in line with estimates from around the world (30%-50%) (4, 26-28), but much higher than what has been reported in Asia

(36.3%) (29), the United States (31%) (30), and China (35%) (31). This would indicate the result is unique to the Arab population. Furthermore, this incidence rate is very similar to what has been reported from Europe (Italy (43%) (32), Turkey (44%) (33), and Germany (41%) (34), although one reported study from Mexico indicated an unusually high prevalence of 86% (35).

In terms of KRAS mutation distribution, the current study reflects what has been defined as biological patterns, as KRAS exon 2 mutations, primarily mutations at codons 12 and 13 (41.89%) (36, 37). These KRAS exon 2 mutations have been shown to induce activation of the Ras/MAPK pathway, which contributes to tumourigenesis and subsequent resistance to anti-EGFR therapy (i.e., cetuximab and panitumumab) (38, 39). On the other hand, KRAS exon 3 and exon 4 mutations (e.g., codons 59, 61, 117, 146) occur less frequently (1%–5%) and are well characterized across the globe (35, 40). In particular, KRAS codon 61 mutations have been increasingly linked to secondary resistance to anti-EGFR therapy and could be detected in the ctDNA of up to 50% of patients (41).

The three dominant mutations at the level of amino acid were p.G12D, p.G12V, and p.G13D, which corresponded to worldwide trends (4). There was a very low prevalence of clinical-associated mutations (e.g., p.G12C, p.A146P/T/V, and codon61 substitutions), all of which have the potential to affect the answers to treatment and prognosis of Certified Registered Geneticists working with patients who have these mutations.

The overall unadjusted proportion of all BRAF mutations in the database was 5.08%. Crucially, our trim-and-fill analysis suggested publication bias, leading to an adjusted prevalence of 8.5%. This adjusted value suggests that BRAF mutations may be under-reported in the region and the true rate may be closer to that seen in and Germany (7%) (34), and slightly higher than Seen in Asia was 5.6% (29), Mexico (6%) (35), while Italy's was (11.1%) (32) and China (16%) (31). The high incidence of the V600E variant as being indicative of poor prognosis, especially among patients diagnosed with stage II-III CRC (42). However, the limited number of BRAF-focused studies and small sample sizes warrant cautious interpretation.

It is worth mentioning that some of the included studies showed extreme outlier values such as 0% prevalence for KRAS or up to 51.85% for BRAF . These abnormal figures do not reflect the real biological frequency of these mutations in the region. Instead, they are likely to be due to the very small sample sizes in those particular hospital-based reports. Therefore, these extreme values should be interpreted with caution and our pooled meta-analysis provides a more realistic picture.

Significant variability was observed based on (I^2 =89.3% for KRAS) and Methodological/geographical differences. Studies that used Sequencing detected the highest KRAS prevalence (53.57%) followed by those using (48.55%) and those using both techniques at the same time (41.75%) (p=0.3551). Sequencing allows researchers to identify both common and rare variants of KRAS, whereas PCR may not identify new or low-frequency variants. The geographical differences identified were statistically significant (p=0.0218), with Libya having the highest KRAS mutation rate at (52.07%) and Lebanon with the lowest at (38.14%). This variability in KRAS mutations could potentially be due to differences in diagnostic protocols, biological characteristics of tumors, as well as differences in genetic makeup of populations, in conjunction with inconsistent reporting standards.

In contrast, it is interesting to note that countries with a higher prevalence often used smaller, local cohorts, which may increase the mutation rate versus larger, national level registries. The data is also heavily skewed toward Egypt, Iraq, Saudi Arabia and Morocco, which contributed most of the sample size. Thus, the observed regional differences could be due to local screening tools and environmental factors specific to these different populations rather than a shared genetic profile. Hence these findings should be considered as a comparison between specific regional cohorts rather than a definitive profile of the entire Arab world.

Although the Meta-analysis consisted of 22 countries in the Arab League, gene mutation (KRAS) data was collected from only 9 countries, while BRAF was only collected from 7 countries. This demonstrates that only a small number of Arab League countries are geographically represented in this statistical analysis. Most of the data came from four countries (Egypt, Iraq, Saudi Arabia and Morocco) to the exclusion of Sudan, Qatar, Yemen and Somalia, who were poorly represented in terms of geographic coverage. As a result, the lack of geographic diversity limits generalizability and highlights the need for collaborative research efforts among the various countries of the Arab League. Additionally, most of the important clinical characteristics of patients, such as tumor stage, histology type, patient age, and microsatellite instability (MSI), were not systematically documented in all cases, which are important factors in the assessment of the prevalence of mutations. Therefore, future studies should systematically record these clinical factors. The vast implications of the above-mentioned findings affect how we treat patients today. According to recommendations issued by the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO), only patients with wild-type KRAS should be prescribed anti-epidermal growth factor receptor (EGFR) inhibitors (38, 39). All mutations at codons 12 and 13 have predicted resistance to anti-EGFR therapies. It is vitally important that KRAS testing is performed prior to initiation of a targeted therapy so that we do not expose patients to unnecessary or avoidable toxicity and costs as well as prescribed ineffective therapies (2).

BRAF mutations occur infrequently; however, BRAF mutations (particularly V600E) have been linked to an aggressive disease course and poor prognosis. Therefore, BRAF testing is recommended for all patients with metastatic colorectal cancer (CRC) according to many international guidelines. V600E mutation status indicates the potential benefit of combined treatment with BRAF inhibitors (e.g., encorafenib) and an anti-EGFR agent, both of which have been shown to significantly improve overall survival outcomes. Further, BRAF status helps determine prognosis and can potentially impact treatment decisions regarding immunotherapy (42).

The findings of this study strongly suggest that molecular profiling such as KRAS and BRAF testing should be an

integral part of Standard of Care for patients diagnosed with CRC in the Arab region. Increasing availability of high-quality diagnostic tests, educating healthcare professionals and including molecular information into national treatment protocols are key to introducing Precision Oncology into the Arabic World.

This meta-analysis also provides evidence-based information to assist in Regional Health Policy Development. As part of a Routine Care Protocol, it is important that all patients receive Routine Mutation Testing prior to receiving treatment in Tertiary Care Facilities. To provide appropriate treatment stratification and resource allocation, investment into Laboratory Capabilities, Quality Assurance and the establishment of a National Cancer Registry with Molecular Data is needed. Cooperation between Regional and International Partners is critical to the establishment of an equitable and sustainable framework that meets the clinical and financial needs of the Arab Healthcare System.

The findings regarding the current meta-analysis should be done in conjunction with the Systematic Review published by Benmokhtar et al., 2024). When compared to this systematic review the two studies identified that the frequency of KRAS mutations amongst patients diagnosed with Colorectal Cancer in this region is much higher than that of BRAF mutations. However, through performing quantitative synthesis of prevalence estimates, evaluating heterogeneity, and assessing publication bias the authors have therefore enabled their research to provide a more complete epidemiologic evaluation of KRAS and BRAF mutation patterns within available Arab cohorts of Colorectal Cancer.

This review was primarily about KRAS and BRAF mutations since those two markers were the most frequently reported molecular abnormalities found in all the studies reviewed. NRAS mutations, HER2 amplification, microsatellite instability status, and circulating tumor DNA (liquid biopsy) were clinically relevant but not reported frequently enough to allow for a quantitative synthesis of these markers across the studies reviewed. Future studies performed regionally should include all of the above markers in order to provide a more complete molecular characterization of colorectal cancer occurring in Arab populations.

Limitations:

There are many key limitations to this meta-analysis. To begin with, all of the studies included in this meta-analysis have limited geographic representation. The Arab world, which is made up of 22 countries, only had 9 of those countries represented in the KRAS analysis and 7 of the 22 countries represented in the BRAF analysis. The majority of these studies were from Egypt, Iraq, Saudi Arabia and Morocco. Many countries in the Arab world do not have representation in this meta-analysis, including: Sudan, Yemen, Qatar, and Somalia. Because of this limited regional representation, it will be difficult to generalize the results of this meta-analysis to the entire Arab world and the findings may not be able to accurately reflect the differences that exist between the respective regions regarding mutation prevalence caused by genetic diversity, environmental exposures, and disparities between healthcare systems. In addition to the issues with geographical representation, there was an extremely large degree of heterogeneity among the studies included in this analysis; the heterogeneity was very high (I^2 = 89.3% KRAS) and this can lead to a decreased confidence in the pooled estimates. This heterogeneity can be attributed to methodological differences across studies; for example, studies used a variety of molecular detection methods (PCR, sequencing, and hybrid approaches) that have differing levels of sensitivity/specificity resulting in significant differences in the level of heterogeneity observed between the studies. There were many inconsistencies in the reporting standards used by the studies, the sample size used in the studies, and a great number of studies did not provide any complete or comprehensive documentation for the clinical and demographic data collected from patients included in these studies. Because of these inconsistencies, it was not possible to conduct a more detailed subgroup analyses of the studies, which will limit the potential to identify patterns of cumulative mutation prevalence above and below the norm within the respective populations. It is also critical for those interpreting these results to keep in mind that the level of heterogeneity observed makes interpretation difficult.

PRISMA 2020 guidelines were adhered to in this review despite it not having been prospectively registered in PROSPERO or any systematic review registry. The potential for methodological bias due to lack of prior registration should also be considered when interpreting the results of this report.

Recommendation for Standardization of Molecular Testing:

Other important contributors to the high level of heterogeneity observed in this publication are the differing methods, approaches, and techniques of molecular testing utilized by each of the contributing studies. Differences in utilization of PCR-based, sequencing-based, or combined types of detection techniques created a variation in prevalence rates for mutations that made it difficult to compare and interpret the findings reported by the different studies. To assist in this process, we strongly suggest implementing standardized procedures/protocols for KRAS and BRAF mutation testing between all research and clinical laboratories operating within the Arab region. Adopting internationally accepted standards/protocols created by organizations such as the College of American Pathologists (CAP), the Association for Molecular Pathology (AMP), and the European Society for Medical Oncology (ESMO) and adapting them for local use, will assist laboratories to standardize their sample collection and preparation procedures, assay sensitivity, mutation reporting format, and quality control procedures. Standardization of molecular testing methods and techniques will reduce the number of differing methods used to perform molecular testing, improve confidence in the accuracy and reproducibility of genomic data, and support comparability between studies. In addition, having harmonized protocols will enable collaboration within the region, facilitate data sharing between laboratories, and improve adoption of personalized oncology within the routine care of patients diagnosed

with colorectal cancer. Therefore, future multicenter studies should focus on the use of validated and consistent methodologies for generating high-quality, comparable, and meaningful clinical data regarding the genomics of CRC in Arab populations.

Conclusion

These results of the meta-analysis show the large number of colorectal cancer patients with KRAS mutations . BRAF mutations were first identified at low levels, but correcting for publication bias suggests they are underreported in the region, highlighting the growing clinical importance of this mutation. Given this strong affiliation between KRAS and colorectal cancer, there is now a clear requirement for routine, standardized molecular profiling of all patients with colorectal cancer in the represented Arab countries to allow for optimal use of targeted treatments and improve patient outcomes. The findings also identified a large amount of methodological variability across the studies included in the meta-analysis and limited data from many Arab countries, which together result in limitations to the generalizability of the findings and the identification of important gaps in the area of cancer genomics in the Arab region. In order to address these limitations, it is critical that the Arab region establishes harmonized protocols for molecular testing of all patients with colorectal cancer and increase the number of multicenter studies conducted in underrepresented countries; and also develops a comprehensive dataset of molecular information for each pharmaceutical agent in the molecular profiling dataset available for each country, which can then be integrated into the national cancer registry in each Arab country. Additionally, future research should focus on closing geographic and methodological gaps between studies, identifying other molecular abnormalities found in Arab colorectal cancer patients, and developing precision oncology approaches that are specific to the Arab context. Pursuing these priorities will provide the necessary foundations to ensure that all colorectal cancer patients in the Arab region receive equitable, evidence-based care and have access to the most effective, customized treatment options available.

Declarations

Ethics approval and consent to participate Ethical approval for this study was obtained from the Iraqi Ministry of Health, Baghdad Medical City, under approval letter No. 30918, dated 17 August 2023.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the NCBI GenBank repository under the following accession numbers:

KRAS sequences: PV883070, PV883071, PV883072, PV883073, PV883074, PV883075, PV883076.

BRAF sequences: PV883077, PV883078, PV883079, PV883080, PV883081, PV883082, PV883083.

These data can also be accessed by searching the accession numbers in the NCBI Nucleotide database.

Competing interests

The authors declare no conflicts of interest.

Funding:

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Authors' Contributions:

T.A.K. was the first author and led the study design and proposal development. She conducted all practical laboratory work, including sample collection, and the development of novel PCR and primer protocols. She also prepared both the initial and final drafts of the manuscript. S.A.E. performed statistical data analysis and contributed to the interpretation of results. S.B.M., as co-supervisor of the study, conducted the bioinformatics analysis, submitted data to NCBI, and contributed to drafting and revising the manuscript. R.S.M., also a co-supervisor, contributed to the histopathological procedures and laboratory supervision. M.F.R. served as the principal supervisor, overseeing all aspects of the project and contributing to the writing and revision of the manuscript. All authors reviewed, provided critical feedback, and approved the final version of the manuscript.

Supplementary Data:

Supplementary Fig 1Mutations_categorization
Supplementary File 1 search queries
Supplementary File 2 All screened papers
Supplementary File3 BRAF Full data
Supplementary File 4 KRAS Full data
Supplementary Fig 5_forest plots for KRAS Exons, codons and Amino acid alterations
Supplementary Fig 6 Forest plot of KRAS Mutation Prevalence by location methods and study period
Supplementary Fig 7_forest plots for BRAF Exons, codons and Amino acid alterations
Supplementary Fig 8 Forest plot of BRAF Mutation Prevalence by location methods and study period

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