Systematic Review and Meta-analysis of TP53, HER2/ERBB2, KRAS, APC, and PIK3CA Genes Expression Pattern in Gastric Cancer

Morteza Ghojazadeh[1], Mohammad Hossein Somi[2], Amirreza Naseri[3,4], Hanieh Salehi-Pourmehr[1], Sina Hassannezhad[1,4], Arash Hajikamanaj Olia[1,4], Leila Kafshdouz[5], Zeinab Nikniaz[2*]

[1]Research Center for Evidence-based Medicine, Iranian EBM Centre: A Joanna Briggs Institute (JBI) Center of Excellence, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[5]Genetic Department, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

*Corresponding Author:
Zeinab Nikniaz, PhD
Liver and Gastrointestinal Diseases Research Center,
Tabriz University of Medical Sciences,
Tabriz, Iran
Tel: +98 4133367473
Fax: +984133367473
Email: Znikniaz@hotmail.com

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Abstract

Background:
With a global prevalence of about 10%, gastric cancer is among the most prevalent cancers. Currently, there has been an ongoing trend toward investigating genetic disruptions in different cancers because they can be used as a target-specific therapy. We aimed to systemically review some gene expression patterns in gastric cancer.

Methods:
The current systematic review was designed and executed in 2020. Scopus, PubMed, Cochrane Library, Google Scholar, web of knowledge, and Science Direct were searched for relevant studies. A manual search of articles (hand searching), reference exploring, checking for grey literature, and seeking expert opinion were also done.

Results:
In this review, 65 studies were included, and the expression pattern of HER2/ERBB2, ER1/Erb1/EGFR, PIK3CA, APC, KRAS, ARID1A, TP53, FGFR2 and MET was investigated. TP53, APC, KRAS, and PIK3CA mutation cumulative frequency were 24.8 (I^2=95.05, Q value=525.53, df=26, P<0.001), 7.2 (I^2=89.79, Q value=48.99, df=5, P<0.001), 7.8 (I^2=93.60, Q value=140.71, df=9, P=0.001) and 8.6 (I^2=80.78, Q value=525.53, df=9, P<0.001) percent, respectively. Overexpression was investigated for HER1/Erb1/EGFR, PIK3CA, APC, KRAS, ARID1A, TP53, CCND1, FGFR2, MET and MYC. The frequency of TP53 and HER2/ERBB2 were 43.1 (I^2=84.06, Q value=58.09, df=9, P<0.001) and 20.8 (I^2=93.61, Q value=234.89, df=15, P<0.001) percent, respectively.

Conclusion:
More research is encouraged to investigate the genes for which we could not perform a meta-analysis.

Keywords:
Gastric cancer, Over expression, Systematic review

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Introduction

Gastric cancer (GC) is among the most common gastrointestinal cancers, and it is the top cause of cancer-induced death in many countries such

as Iran, Kyrgyzstan, and Chile.[1,2] The lowest estimated mortality rates are in Northern America, and the highest are in Eastern Asia.[3] Genetic and environmental differences may be related to the geographical variances in GC frequency.[4] Like other cancers, GC is caused by specific changes in genes that affect the cell's ability to grow and divide appropriately.[5] Determination of the genetic basis of GC can help better understand its pathogenesis, identify new biomarkers and new target-specific treatments, and help clinicians estimate GC prognosis.[6] However, little is known about which genetic events are essential in gastric carcinogenesis. Numerous genetic and epigenetic changes in tumor-suppressor genes, DNA repair genes, genetic instability, and oncogenes cell adhesion molecules are implicated in the carcinogenesis of the stomach.

According to recent classifications, GCs are sorted into four subtypes: Microsatellite instability (MSI), Epstein-Barr positive, Chromosomally unstable mutation, and Chromosomally stable subtypes.[7] In human cancers, including GC, the most common genetic alteration is p53 mutation.[8] The Epstein-Barr positive subtype, which mostly bears mutations in the PIK3CA gene, is the second most observed mutation in many cancers.[9] *PIK3CA* mutations are present in 32% of patients with hypermutated tumors and 9%-12% of non-hypermutated tumors. Its mutation in GC is frequently seen in EBV positive and MSI subtypes, despite the unclear pathological or clinical implications.[10] JAK2, CD274, and PDCD1LG2 gene amplification have also been observed in Epstein-Barr positive GC cases and deletions in CDKN2A, SMAD4 PTEN, and ARID1A.[11] The HER2 known as HER2/neu or ERBB2 encodes receptors of transmembrane tyrosine kinase. It is expressed in several tissues physiologically; however, when its related pathway activates uncontrollably, disproportionate cell growth, tumorigenesis, and angiogenesis happen. Studies showed that excess gene amplification and overexpression of HER2 are linked with multiple solid tumors such as GC.[12] Carcinogenesis of GC involves mutations in adenomatous polyposis *coli* or APC, which is independent of the MSI phenotype.[13]

Generally, in MSI subtype mutations in EGFR, PIK3CA, ERBB2, and ERBB3 accumulate.[11] The chromosomally unstable subtype involves mutations in TP53, followed by mutations in KRAS, ARID1A, PIK3CA, ERBB2, APC, and RNF43 genes.[14] The chromosomally stable subtype is characterized by CDH1 and ROHA mutation besides ARID1A inactivation.[15]

As discussed above, many genetic predisposing changes are recognized for GC, and different studies have been conducted to assess the gene expression pattern in GC patients. However, no systematic review or meta-analysis has been conducted to summarize the results. Therefore, in the present study, we conducted a systematic review and meta-analysis of the frequency of different patterns of gene expressions in patients with GC.

Materials and Methods

Study Design and Search Strategy

We searched Web of Science, Scopus, Cochrane Library, Medline (Ovid), and Embase for documents published up to January 2020. Keywords were selected using Mesh terms and following keywords among others: "gastric cancer*", "gastric neoplasms", "stomach cancer*", "stomach neoplasms", "stomach carcinoma*", "gastric carcinoma*", "Gene", "amplification", "Mutation", "expression", "Genetics", "prevalence". The bibliographies of included articles were also searched for related articles not identified by electronic search. The research protocol of the present study is approved by the Research Center for Evidence-Based Medicine of Tabriz University of Medical Sciences. The PRISMA statement was utilized to design the current systematic review and meta-analysis.

Study Selection

This review included observational studies published in English from January 1980 to January 2020 as a journal article or conference paper and evaluated gene expression patterns in GC. Experimental investigations and studies without sufficient sample size or quality were excluded.

Data Extraction

Data extraction was done independently by two reviewers. At first, one author screened the identified records to remove irrelevant or duplicated manuscripts. The remaining records were examined independently by two reviewers to identify articles meeting the

inclusion criteria. The full-text version of these records was retrieved and separately assessed by two authors. One author extracted the data on the publication date, authors, sample size, method, and studied outcomes for each included article. A second reviewer checked the results of the data extraction. Two reviewers discussed inconsistencies and resolved them. by referring to a third reviewer. Five articles were chosen after the critical appraisal of articles (Figure 1).

Quality Assessment

Two reviewers independently used Newcastle-Ottawa Scale to rate the methodological quality of the included studies. The third investigator resolved the discrepancies between the two raters.

Statistical Analysis

Analyses of data were done using the Comprehensive Meta-Analysis version 2.0. heterogeneity among studies was assessed through Q and I^2 statistics. In this meta-analysis, a significance level of $P<0.10$ for Cochran's Q and $I^2>50\%$ defined clinically significant heterogeneity.[16] Given the results of heterogeneity analysis, a random-effect or fixed-effect model was used. A P value less than 0.05 was considered statistically significant.

Results

Of 864 identified studies, 65 were included in the systematic review. The flowchart of the review is displayed in Figure 1. The characteristics of included studies in terms of gene expression patterns are shown in Table S1 and Table S2,[17-81] respectively.

The Pattern of Genes Expression in Included Studies

Forty studies evaluate various gene mutations in patients with GC. Seven studies were done in China,

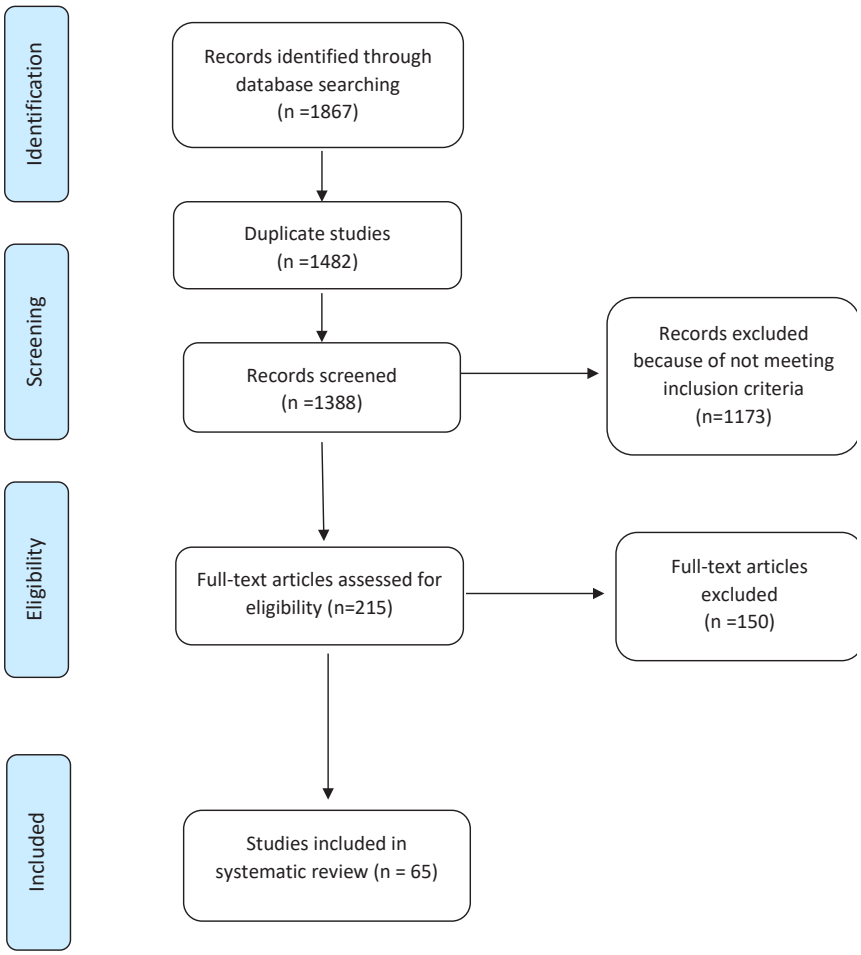

Figure 1. Flowchart of the systematic review process reviewing studies investigating gene overexpression and mutation in gastric cancer.

four in Iran, three in Italy, and 26 in other countries, including Singapore, the UK, Slovenia, Germany, Japan, Korea, Taiwan, Saudi Arabia, and the USA. From the included articles, data about mutations in KRAS, APC, PIK3CA, HER1/Erb1/EGFR, HER2/ERBB2, ARID1A, TP52, FGFR2 and MET was extracted. Due to the lack of studies, a meta-analysis was only performed for KRAS, TP53, APC, and PIK3CA mutations (Table 1). TP53, APC, KRAS and PIK3CA mutation cumulative frequency were 24.8 (I^2=95.05, Q-Value=525.53, df=26, *P* – value<0.001), 7.2 (I^2=89.79, Q-Value=48.99, df=5, *P* value<0.001), 7.8 (I^2=93.60, Q-Value=140.71, df=9, *P* value=0.001) and 8.6 (I^2=80.78, Q-Value=525.53, df=9, *P* value<0.001) percent, respectively. The heterogeneity in these studies was high, so the random effect was used. The forest plot of studies assessing TP53 mutation in GC is shown in Figure 2.

Of 30 included studies for gene over-expression, five were conducted in South Korea, eight in Japan, two in Brazil, and the remained studies in the USA, China, Slovenia, Portugal, Turkey, and Iran. Overexpression of CCND1, TP53, ARID1A, APC, PIK3CA, HER1/ERB1/EGFR, FGFR2, MET, and MYC were extracted from included studies. In the study conducted on the Iranian population, Azarhoosh et al studied the overexpression of TP53 in two groups of cardia and antrum adenocarcinoma, so it is considered two different populations. Also, the study of Kuboki et al in Japan was considered two different population, too. Due to the lack of studies, a meta-analysis was only performed for HER2/ERBB2 (17 studies) and TP53 (10 studies) (Table 1); The frequency of TP53 and HER2/ERBB2 were 43.1 (I^2=84.06, Q-Value=58.09, df=9, *P* value<0.001) and 20.8 (I^2=93.61, Q-Value=234.89, df=15, *P* value<0.001) percent, respectively. The forest plots for TP53 and HER2/ERBB2 are shown in Figure 3.

Discussion

Genetic changes in cells are the key event in cancerous cells formation.[17] The changes in sequences managing the cell cycle or apoptosis, protein synthesis, and cellular transduction are the most common genetic changes which bring a new phase of development in the survival of cancerous cells.[82] So, knowledge of these genetic changes improves recognition of malignancy's clinical and pathological behavior and sheds light on a new method for cancer treatment.[83]

In this systematic review and meta-analysis, the most studied gene was TP53. The loss of TP53 function as a tumor-suppressing gene is proposed to have a grave role in tumorigenesis.[84] This change makes the cancerous cells more influenced by DNA changes so that the cell does not undergo apoptosis when a critical change is made in DNA.[84] In carcinogenesis of GC, TP53 mutation is an early event in gastric adenocarcinoma formation, which turns intestinal metaplasia into GC.[85] However, unlike TP53 mutation, the TP53 expression is an event also seen in intestinal

Table 1. Gene mutation and overexpression in gastric cancer included in the meta-analysis

Model			Effect size and 95% interval				Test of null (2-Tail)		Heterogeneity			
			Number studies	Point estimate	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	*P* value	I-squared
Expression	Random effects	TP53	10	0.431	0.350	0.517	-1.571	0.116	58.09	9	<0.001	84.06
	Random effects	HER2/ERBB2	16	0.208	0.143	0.295	-5.752	<0.001	234.89	15	<0.001	93.61
Mutation	Random effects	KRAS	10	0.078	0.034	0.169	-5.513	<0.001	140.71	9	0.001	93.60
	Random effects	APC	6	0.072	0.022	0.211	-4.047	<0.001	48.99	5	<0.001	89.79
	Random effects	PIK3CA	10	0.086	0.061	0.119	-12.64	<0.001	46.81	9	<0.001	80.78
	Random effects	TP53	27	0.248	0.172	0.343	-4.74	<0.001	525.53	26	<0.001	95.05

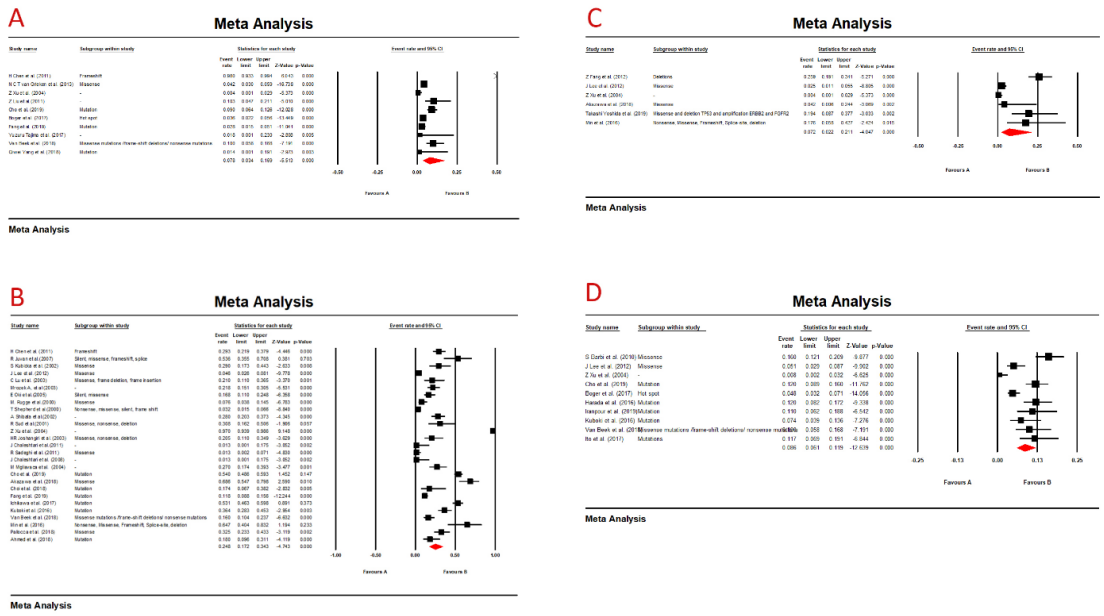

Figure 2. The forest plot for the studies investigating KRAS (A), TP53 (B), APC (C), and PIK3CA (D) mutation.

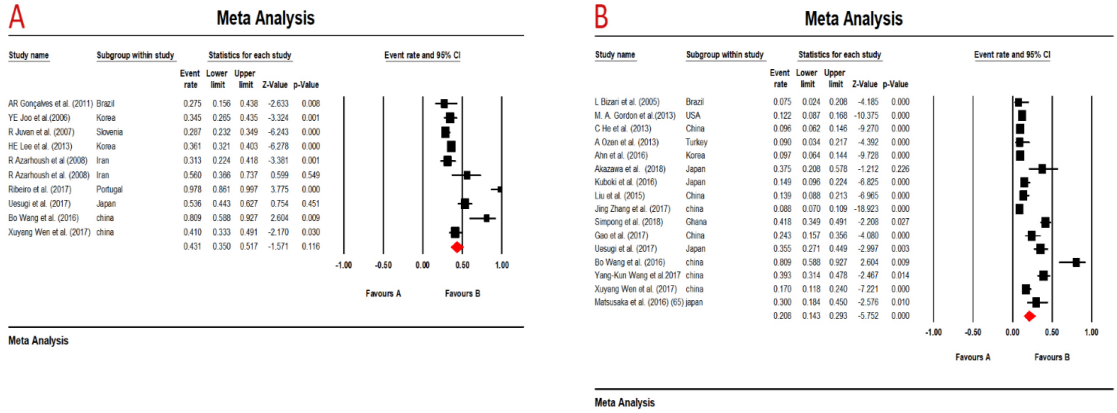

Figure 3. The forest plot for the studies investigating TP53 (A) and HER_2/$ERBB_2$ (B) over-expression.

metaplasia.[86] It is thought that TP53 over-expression is the main event when normal gastric mucosa turns into intestinal metaplasia and then GC.[8] In the present study, the frequency of TP53 mutation and over-expression in all GC patients, regardless of their subtype, was 24.8% and 43.1%, respectively. Previous studies have shown that TP53 mutation and expression are events mostly seen in adenocarcinoma and intestinal subtypes of GC and less prevalent in a diffuse subtype of GC. So, the differences between studies regarding the frequency of TP53 mutation and overexpression may be related to the histological subtypes GC in studied samples.

HER2/ERBB2 is a proto-oncogene, encoding a transmembrane tyrosine kinase, leading to cell differentiation and growth.[87] HER2/ERBB2 over-expression has been identified as an early event in gastric adenocarcinoma development.[88] The prognostic value of HER2/ERBB2 over-expression has always been a subject of interest for researchers; in most studies, it has been reported that this amplification is correlated with poor prognosis.[24] An essential aspect of HER2/ERBB2 over-expression is the possibility of using a monoclonal antibody targeting this amplification named Herceptin.[89] This treatment has decreased the mortality rate among those with positive HER2/ERBB2 amplification.[90,91] In this systematic

review, the frequency of HER2/ERBB2 was 20.8%. Like TP53, HER2/ERBB2 overexpression is mostly seen in the intestinal subtype of GC

KRAS is a protein involved in regulating cell division through mitogen-activated protein kinase, which is among the main intracellular pathways affected in carcinogenesis.[92] KRAS mutation is seen in about 30% of human cancers.[93] KRAS mutation, bypassing the epidermal growth factor signal pathway, reduces the susceptibility of cancerous cells to anti-HER2 target therapy.[94] The efforts to target KRAS protein in cancerous cells have been inconclusive so far, so some downstream proteins to RAS or parallel pathways such as the PI3K pathway, of which PIK3CA is a part, have been chosen as the strategies to inhibit cancerous cell function.[95] In this review, the frequency of KRAS and PIK3CA mutations were 7.8% and 8.6%, respectively. This is far less than what has been reported about other gastrointestinal tract malignancies which KRAS and PIK3CA mutations are seen in about 27-37 and 20-32 percent of them[96-98] this difference might be due to the different pathologic process going on in gastric malignancies, also explains the different pathophysiological scenarios happening in GC.

APC is a tumor suppressor gene in the WNT signaling pathway, considered a "gatekeeper" for many neoplastic transformations in the gastrointestinal system.[99] APC mutation is considered an early event in neoplastic transformations in the gastrointestinal system.[94] This mutation was first identified as familial adenomatous polyposis syndrome, in which individuals were susceptible to colon cancer. In present study, the frequency of APC mutation was 7.2 (I^2=89.79, Q-Value=48.99, df=5, *P* value<0.001) with a high heterogeneity in included studies. In one of the included studies, Fang et al reported that APC mutation was seen in about 25 percent of 131 patients with gastric adenocarcinoma, where no subtype categorization was performed.[39] While, Xu et al had concluded that APC mutation frequency was 0.4 percent, where GC subtype categorization was also done.[32] This difference might be due to the different exons being investigated for APC mutation, although the investigated exons were not mentioned. In a study by Fang et al about the APC mutation in gastric carcinoma, it was concluded that the frequency of APC mutation in intestinal and diffuse-type gastric carcinoma were 33.3% and 13.1%, respectively; it also was mentioned that there might have been an underestimation of APC mutation frequency because only exon 15 of APC was investigated for mutation.[13]

Limitations of this study include the type of selected studies (observational studies), the heterogeneity found between the results of included studies, and the differences in the sample size of studies. Moreover, as a limitation of this study, no categorization was performed to evaluate different genetic changes in each subtype. These limitations undoubtedly produced some analytical and measurement bias in this meta-analysis.

The present study determined the expression pattern of TP53, HER2/ERBB2, APC, KRAS, and PIK3CA in GC. The slight difference between the frequency of these genetic changes in GC and other gastrointestinal malignancies proposes the different pathological processes and subsequent treatment needed for GC. These results manifest the significance of target therapies in treating GC. Further studies are needed for different GC subtypes to provide precise information on this matter.

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Ethical Approval

Not applicable.

Conflict of Interest

The authors declare no conflict of interest related to this work.

Supplementary Files

Supplementary file 1 contains Tables S1 and S2.

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