Diagnostic Value of microRNAs for Gastric Carcinoma: a Meta-Analysis

Zheng Han, Yifu Zhang, Huixia Zhu*

Department of Clinical Medicine, Medical School of Nantong University, Nantong, Jiangsu, 226001, China
*Corresponding author: hanzhengzhx@163.com

Abstract: Purpose: To systematically evaluate the potential diagnostic value of MicroRNA for gastric cancer (GC). Methods: The relevant literature was identified in databases such as PubMed, Embase and the Cochrane Library (up to December 25, 2020). Two researchers independently selected the literature based on the inclusion and exclusion criteria, extracted data, and evaluated the risk of bias. Review Manager 5.4, Meta-Disc 1.4 and STATA (version 15.1) software were performed the Meta-analysis. Results: A total of 5914 patients from 41 studies were ultimately included. The pooled sensitivity (SENS) was 0.79 (95% CI: 0.75–0.82), the pooled specificity (SPEC) was 0.87 (95% CI: 0.82–0.91), the pooled positive likelihood ratio (PLR) was 4.95 (95% CI: 3.81–6.43), the pooled negative likelihood ratio (NLR) was 0.28 (95% CI: 0.24–0.32), the pooled diagnostic odds ratio (DOR) was 20.53 (95% CI: 14.57–28.94), and the area under the curve (AUC) was 0.87 (95% CI: 0.84–0.90). A Deeks’ funnel plot demonstrates no publication bias existed (P=0.40). Meta-regression analysis showed that sample size, sample source and sample type were potential sources of heterogeneity. Conclusions: MicroRNA might be the potential biomarker diagnosing gastric cancer.

Keywords: MicroRNA; gastric cancer; diagnosis; Meta-analysis

1. Introduction

Malignant tumor is a worldwide public problem, among which gastric cancer (GC) is the sixth most common cancer and the fourth leading cause of cancer-related death in the world (1-3). Unfortunately, a majority of GC patients have been at a progressive stage when they were confirmed diagnosis, owing to lacking sensitive biomarkers for early-stage GC. A number of studies have revealed that the 5-year survival rate of patients with early GC can reach 90%, however, for patients with advanced GC, the median survival time was only 6-9 months (4). Therefore, it is crucial to obtain an efficient diagnosis to raising the 5-year survival rate. Currently, endoscopy has been widely used in the diagnosis of GC, but it still has limitations due to its invasive nature and relatively high costs (5). Therefore, bio-markers which can be stably detected in cell free body fluids, such as serum or plasma, are the key to reducing the mortality rate and improving the prognosis of people in early stage of GC.

To create a non-invasive and low-priced method, bio-marker detection have been widely used in the diagnosis of GC. However, methods for the detection of carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), and carbohydrate antigen 724 (CA724) lack adequate sensitivity and specificity to distinguish aggressive from indolent tumors which has precluded their widespread application in early diagnosis of GC (6).

MicroRNAs is a class of evolutionarily conserved and 22nt non-coding RNA molecules that plays roles in regulating gene transcription and expression via multiple pathways, and in physiological processes such as cell cycle and senescence. The expression profile of miRNAs in GC patients usually exhibits exceptionally high in contrast to that in normal specimens (7). It is reported that MicroRNAs can be stably detected in serum or plasma and remain stable after up to eight cycles of freeze-thawing or after incubation at room temperature for up to 24h. Compared with other biomarkers, their stability and easily testable length (about 22 bp) make MicroRNAs well suited to be effective, non-invasive, novel and operable GC biomarkers.

In 2008, Mitchell et al first reported that expression levels of microRNAs were significantly abnormal in the GC tissue, as compared to the unaffected controls (8). Recently, several studies have shown that microRNAs are highly specific in the diagnosis of GC (9, 10). In particular, it has a very high sensitivity
for cases of GC, suggesting that microRNAs are helpful for the early diagnosis of GC (11, 12). Numerous studies demonstrated that microRNAs may be a potential non-invasive molecule for GC, but with varying diagnostic accuracy (13-19). In the present meta-analysis, we included 41 studies involving miRNA expression profiling to systematically and comprehensively evaluate the diagnostic efficacy of microRNAs for GC through quantitative Meta-analysis, and then provide a scientific basis for clinical guidance.

2. Methods

The PRISMA statement (S1 PRISMA Checklist) was followed in this meta-analysis. The study protocol was registered with the PROSPERO international prospective register of systematic reviews (registry number CRD42020214532).

2.1. Literature search

Two authors (HZ and ZYF) independently searched PubMed, Embase and the Cochrane Library to identify potentially eligible studies published before December 25, 2020. The keywords used for literature retrieval were (‘microRNA’ or ‘miR’ or ‘miRNA’) and (‘gastric cancer’ or ‘gastric tumor’ or ‘gastric carcinoma’ or ‘gastric neoplasm’) and (‘diagnostic’ or ‘diagnosis’ or ‘sensitivity and specificity’ or ‘ROC curve’) and (‘circulating’ or ‘serum’ or ‘plasma’ or ‘blood’). Citations of review articles and identified articles are also studied. All publications identified by our search strategy were independently evaluated by two reviewers. Any disagreement on a controversial study was resolved by discussion to consensus.

2.2. Eligibility criteria

All studies included in the meta-analysis meet the following criteria:
① All cases were confirmed by pathological examination;
② The study explored the correlation between GC levels and MicroRNA expression diagnosis;
③ Studies should contain the data of specificity, sensitivity (or the possibility of deriving such values from the data);

Publications were excluded if they got any of the following items:
① The subjects of the literature were animals, not humans;
② Letters, editorials, meeting abstracts, case reports and reviews;
③ Studies lacking sufficient data to construct a diagnostic 2 × 2 table;

2.3. Data extraction and quality assessment

The following patients’ characteristics were collected for each study: the first author’s name, publication year, country, specimen, sample size, specificity, sensitivity and area under the curve (AUC), etc. Any disagreement among researchers was resolved through discussions with a third researcher (ZHX) until a consensus was reached.

Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) was appraising the risk of bias and applicability of the included studies using Review Manager 5.4 software. This scale was composed of four domains consisting of patient selection, index test, reference standard, and flow and timing domain. Each signaling question was judged as ‘yes’, ‘no’, or ‘unclear’ and each study's risk of bias and concern for applicability was estimated as ‘high’, ‘low’, or ‘unclear’ except for the flow and timing domain, for which the applicability concern did not apply. An answer of ‘yes’ meant the risk of bias could be judged as being low, whereas an answer of ‘no’ or ‘unclear’ meant that the risk of bias could be judged as being high.
2.4. Statistical analysis

Figure 1: Literature screening process and results

Figure 2: Risk of bias and applicability concern graph.
Statistical analysis was performed using StataSE15.1, Meta-Disc1.4, and Review Manager5.4. Q tests and $I^2$ statistics were used to estimate the heterogeneity caused by a non-threshold effect among the included studies. Either $P<0.1$ or $I^2 >50\%$ suggested the existence of substantial heterogeneity; in this study, a random-effects model was applied to quantify the pooled sensitivity, specificity, PLR, NLR, DOR and AUC. Otherwise, a fixed-effects model was used. Spearman correlation analysis was conducted to verify the threshold effects. Moreover, sources of heterogeneity were explored by meta-regression analysis based on possible characteristics. Sensitivity analysis was performed to assess the stability of our analysis. A Deeks’ funnel plot was performed for evaluating publication bias.

3. Results

3.1. Literature search and selection of studies

The detailed procedure of study selection was presented in Figure 1. A total of 680 articles were systematically retrieved from a primary literature search. First, we roughly screened the titles and abstracts and eliminated 65 publications that were irrelevant to the topic. The remaining 615 articles were further examined by careful review of the full text; as a result, 518 articles were excluded, 5 studies were not considered as they were not human study. Seven studies regarding literature reviews, abstracts and case reports were excluded. After a more detailed evaluation, 44 studies were removed as they did not contain full text or had insufficient data for extraction. Finally, the selection process revealed 41 studies that were eligible for diagnostic analysis.

3.2. Study characteristics

In this study, 41 articles were included, involving a total of 5914 subjects. Among these 41 studies, 21 used serum samples, whereas the rest used plasma. The included studies were performed in China, Iran, Spain, Egypt, Korea, and Japan. Table 1 presents the detailed characteristics of each subject.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Country</th>
<th>Specimen</th>
<th>Bio-markers</th>
<th>Cancer Control Sensitivity</th>
<th>Specificity</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yuntong Guo (20)</td>
<td>2020</td>
<td>China</td>
<td>Serum</td>
<td>miR-296-5p</td>
<td>90 90 84.44%</td>
<td>92.22%</td>
<td>0.9190</td>
</tr>
<tr>
<td>Mona Schaalan (21)</td>
<td>2020</td>
<td>Egypt</td>
<td>Serum</td>
<td>miRNA 200c</td>
<td>50 80 81.20%</td>
<td>100.00%</td>
<td>0.9060</td>
</tr>
<tr>
<td>Huan Ma (22)</td>
<td>2019</td>
<td>China</td>
<td>Serum</td>
<td>miR-647</td>
<td>105 60 80.00%</td>
<td>78.30%</td>
<td>0.8290</td>
</tr>
<tr>
<td>Jie Ning (23)</td>
<td>2019</td>
<td>China</td>
<td>Plasma</td>
<td>miR-138-5p</td>
<td>51 20 79.41%</td>
<td>64.71%</td>
<td>0.7690</td>
</tr>
<tr>
<td>Pegah Parvae (24)</td>
<td>2019</td>
<td>Iran</td>
<td>Plasma</td>
<td>Multiple (miR-107, 194, 210)</td>
<td>50 50 93.80%</td>
<td>78.80%</td>
<td>0.9470</td>
</tr>
<tr>
<td>Jianlin Chen (25)</td>
<td>2019</td>
<td>China</td>
<td>Plasma</td>
<td>miR-421</td>
<td>90 45 96.67%</td>
<td>95.56%</td>
<td>0.9810</td>
</tr>
<tr>
<td>Waleed A. Mohamed (26)</td>
<td>2019</td>
<td>Egypt</td>
<td>Plasma</td>
<td>miR-204</td>
<td>35 40 72.70%</td>
<td>60.00%</td>
<td>0.6880</td>
</tr>
<tr>
<td>Bing Ji (27)</td>
<td>2019</td>
<td>China</td>
<td>Plasma</td>
<td>miR-214</td>
<td>168 74 73.20%</td>
<td>91.90%</td>
<td>0.8800</td>
</tr>
<tr>
<td>Hamid Ghaedi (28)</td>
<td>2018</td>
<td>Iran</td>
<td>Plasma</td>
<td>miR-675-5p</td>
<td>62 42 77.42%</td>
<td>52.50%</td>
<td>0.6610</td>
</tr>
<tr>
<td>Su-yang Bai (29)</td>
<td>2018</td>
<td>China</td>
<td>Serum</td>
<td>miR-551b-3p</td>
<td>50 53 70.01%</td>
<td>96.20%</td>
<td>0.8600</td>
</tr>
<tr>
<td>Gaoping Zhao (30)</td>
<td>2018</td>
<td>China</td>
<td>Plasma</td>
<td>(miR-21, 93, 106a, Multiple)</td>
<td>147 28 88.70%</td>
<td>79.20%</td>
<td>0.8870</td>
</tr>
</tbody>
</table>
3.3. Quality of the Included Studies

QUADAS-2 quality assessment of the included studies and the results of critical appraisal are shown in Figure 2 and Figure 3. Two figures depict the relatively moderate quality of the 41 included studies. Almost all studies had either low or unclear risks of bias due to a lack of information on patient selection, index test, or reference standard.

3.4. Diagnostic accuracy

Heterogeneity might come from either threshold effect or non-threshold effect. The threshold effect was the main cause of heterogeneity, which occurred due to differences in sensitivity/specificity and cut-off value. Heterogeneity among studies was evaluated by examining the threshold and non-threshold effects. In this study, the Spearman correlation coefficient and P-value were 0.658 and 0.218, respectively, indicating that there was no threshold effect. The chart of the ROC curve did not show a “shoulder arm” point distribution also indicates that there was no threshold effect. Heterogeneity owing to non-threshold effects was then assessed with Q-tests and I² statistics.

There was significant heterogeneity in the pooled sensitivity (I² =81.2%, P<0.1) and specificity (I² =88.5%, P<0.1); thus, a random-effects model was applied to analyze the diagnostic parameters. Through meta-regression analysis, we found that sample size, sample source, and sample type were the major potential sources of heterogeneity in this study (Figure 7).

To further explain the heterogeneity of individual studies, we performed a sensitivity analysis by removing individual studies. As shown in Figure 8, 7 studies were identified, which may be the reason for heterogeneity.
Figure 4: Meta-regression.

Figure 5: Sensitivity analysis

Figure 6: Deeks’ funnel plot.
Figure 7: Forest plots of sensitivity and specificity of MicroRNAs for GC diagnosis

Figure 8: The summary receiver operator characteristic (SROC) curve of MicroRNAs for GC diagnosis
3.5. Publication bias

Deeks’ funnel plot asymmetry tests were applied to estimate publication bias of included studies. The slope coefficient was associated with a P value of 0.40, suggesting a low likelihood of publication bias in our meta-analysis (shown in Figure 9).

4. Discussion

GC is responsible for the highest number of cancer-related mortalities, primarily since the majority of patients have a terminal disease at stage III or IV at the time of diagnosis (61). Methods for the detection of CEA, CA199, and CA724 lack adequate sensitivity and specificity to distinguish aggressive from indolent tumors. Compared with other biomarkers, their stability and easily testable length (about 22 bp) make MicroRNAs well suited to be effective, non-invasive, novel and operable GC biomarkers. Based on the present research situation, the present study undertook a meta-analysis to evaluate the diagnostic efficacy of microRNAs for GC.

The pooled outcomes of sensitivity, specificity, and AUC (0.79, 0.87, and 0.87, respectively) with the random effects model revealed that microRNAs have better diagnostic value than CEA and CAA199 (AUC of 0.55 and 0.60, respectively) (Figure 4, 6) in distinguishing GC patients from control groups. The DOR is an index measuring of the effectiveness of a diagnostic test. In this study, the DOR of microRNAs for GC detection was 20.53 (95% CI: 14.57-28.94) (Figure 7). There is heterogeneity among the studies included in this meta-analysis. Meta-regression analysis showed that sample size, sample source, and sample type were potential sources of heterogeneity. A Deeks’ funnel plot demonstrates no publication bias existed (P=0.40). The SROC curve is located near the lower left corner with an AUC of 0.87. All of the data shown above support that microRNAs can be a good indicator for the diagnosis of GC.

Despite our efforts, several limitations should be noted in the meta-analysis. One of the major drawbacks is unpublished and currently being studied data. This may cause publication bias in the study and have a slight impact on the final pooled results. The most obvious disadvantages is that the included studies in the present meta-analysis only distinguished the tumor patients from healthy controls, but other risk factors, such as chronic gastritis, infectious disease, ulcers, and reflux esophagitis, were not included. These factors may contribute to alter miRNA expression. In spite of the limitations mentioned above, this meta-analysis demonstrates a comprehensive assessment and robust evidence that microRNAs have high diagnostic accuracy for assessing GC.
References


