Original article

Role of serum and salivary microRNAs as diagnostic biomarkers in gastric cancer

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Abstract

Introduction: Incidence of Gastric cancer (GC) is increasing alarmingly in the recent past due to changing lifestyle and diet pattern. GC usually has poor prognosis due to delayed diagnosis. Salivary and serum biomarkers are a potential early diagnostic tool for GC. MicroRNAs are promising biomarkers due to their stability in these body fluids and their pivotal role in carcinogenesis. This study helps to determine the role of serum and salivary microRNAs as diagnostic biomarkers in gastric cancer. **Materials and Methods:** The systematic review was performed as per the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. The population, intervention, comparison, outcomes, and study (PICOS) framework was used as a strategy for this review. The data were retrieved from online databases such as Medline (PubMed), Web of Science, Scopus, and SpringerLink. Risk of bias and applicability concerns were evaluated using the four domains of QUADAS-2: patient selection, index test, reference standard, procedure and timing. **Results:** Quality evaluation based on diagnostic accuracy revealed that five studies conducted by Hou *et al.*, Jianhong *et al.*, Kaczor-Urbanowicz *et al.*, Saliminejad *et al.*, and So JBY *et al.* had the lowest risk of bias, according to a risk of bias chart created using Revman 5.4.1 software. A serum 12-miRNA biomarker assay was validated by the low risk of bias research. **Conclusion:** This systematic review provides an insight into the possible role and application of an array of miRNAs from various body fluids as a biomarker for the early detection of gastric cancer. The serum 12-miRNA biomarker assay, validated by a low risk of bias research can serve as a potential diagnostic tool to detect gastric cancer. However, the validation of other salivary, serum, and plasma miRNAs as a diagnostic biomarker for gastric cancer mandates further long-term follow-up studies in diverse ethnicity of populations.

Keywords: *Saliva, Biomarkers, Serum, microRNA, Gastric cancer*

Introduction

Globally, gastric carcinoma (GC) is the second leading cause of cancer-related death and fourth most common gastrointestinal malignancy.[1] GC usually presents with poor prognosis and extremely low survival rate primarily due to delayed diagnosis which can be attributed to nonspecific early symptoms, clinically occult location of the tumor, and unavailability of reliable early noninvasive screening modalities.[2] Invasive endoscopy is the only currently available diagnostic method for GC.

Early detection of cancer is key to good prognosis and improved survival rate. "Liquid biopsy" has made rapid progress and a plethora of biomarkers have been discovered in blood. Saliva has recently emerged as a promising diagnostic tool for various systemic diseases including cancers and is gaining momentum over other body fluids such as blood, as saliva collection is primarily noninvasive and safe. The technique involved in saliva collection is also easy and economic.

MicroRNAs represent a category of highly conserved, short (18–22 nucleotides) noncoding RNAs that are abundantly implicated in tumorigenesis. Studies in the recent past have proved that these miRNAs are stably detectable in plasma and serum.[3] MicroRNAs (miRNAs) are a subclass of short, noncoding RNAs that primarily control the levels of proteins and the

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degradation of mRNA. The origin and precise activities of miRNA have been unraveled in recent years, with an emphasis on their potential applications in both the academic and clinical fields. This review aims to increase current knowledge of these compounds and their potential role in noninvasive detection of gastric cancer. The biological traits and mechanisms of miRNA release that make them interesting candidates as noninvasive gastric cancer indicators are also outlined in this paper.

Scientific Rationale

Increasing evidence suggests that circulating miRNAs could serve as potential Non-invasive diagnostic biomarkers for gastric cancer.[4] Various studies have reported different tissue-associated and circulating miRNA panels as efficient biomarkers for gastric cancer. Since gastric cancer is a fatal disease with poor prognosis, lacks specific early symptoms, requires invasive endoscopy for diagnosis, and often presents with poor prognosis due to its delayed diagnosis and occult anatomical location, these circulating biomarker panels of miRNAs would serve as promising tools for early diagnosis and improved prognosis in these patients. Hence, this systematic review aims at analyzing and compiling the reported panel of miRNAs in plasma, peripheral blood mononuclear cells, serum, and saliva as diagnostic biomarkers in GC.

Materials and Methods

The systematic review was performed as per the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[5] The population, intervention, comparison, outcomes, and study (PICOS) framework was used as a strategy for this review. P – Patients with gastric carcinoma, I – Evaluation of circulating miRNA levels in plasma, saliva and peripheral blood mononuclear cells (PBMNC), C – Circulating miRNAs in normal patients without GC and patients with GC, O – To derive at a noninvasive diagnostic panel of miRNAs for GC, S – To compare miRNA levels in plasma, saliva, and PBMNCs in patients with GC and the levels of these biomarkers in normal patients without GC.

Structured questions were formulated based on the above PICOS strategy:

(1) Are circulating miRNAs efficient, minimally invasive biomarkers for diagnosis of GC?
(2) Can a particular panel of circulating miRNAs be considered as potential diagnostic panel for GC?
(3) To elucidate the potential role of miRNAs in GC.

The following inclusion and exclusion criteria were framed to obtain evidence for the PICOS question:

INCLUSION CRITERIA: A systematic search using the above-mentioned MeSH terms was performed. Selected articles that strictly adhered to the following criteria were considered eligible:

(a) Studies published during the time period of January 2010 to November 2022, (b) articles published in English language, (c) clinical studies (case control/cohort/ randomized control trials), whose content was associated with miRNAs as diagnostic markers of gastric cancer, and (d) *in-vitro* studies that evaluated the role of miRNA in gastric cancer GC.

EXCLUSION CRITERIA: (a) Articles elucidating miRNA extracted from tissue or body fluids like gastric juices, (b) review articles, books, chapters, systematic reviews, and meta-analyses, (c) articles published before or after the specified dates, and (d) studies done without experimental analysis based exclusively only on prediction using bioinformatic approaches. The selection was supplemented by a manual search and screening of the complete reference lists of the included studies. All extracted data were entered into a custom spreadsheet program to eliminate possible errors. Selection of studies is depicted in a flow chart [Figure 2].

A systematic data search was performed and the studies done on human subjects and published in English language describing the use of saliva, serum, and PBMNC biomarkers for screening or diagnosis of GC during the time period of January 2010 to November 2022 were included. The data were retrieved from online databases such as Medline (PubMed), Web of Science, Scopus, and SpringerLink. The review report was based on PRISMA guidelines.[5] The following search terms were used to find records that were accessible online: ((″liquid biopsy*″ OR ″ plasma ″ [Mesh]) OR (saliva OR salivary) OR AND ((Serum* Saliva *OR gastric carcinoma and biomarker). PubMed, Scopus, and the publication date filter were added to this query. No other filters were used.

DATA EXTRACTION: The systematic search yielded both *in-vivo* clinical studies evaluating miRNAs as diagnostic biomarkers for GC and *in-vitro* studies evaluating the role of miRNA in GC.[6-19] Two authors performed the review independently following consultation with the senior author independently. The senior author's independent decision was considered final. All extracted data were entered into a custom Excel spreadsheet to eliminate possible errors. The characteristics of extracted data such as authors, year, samples, type of miRNA evaluated, the type of samples, and outcome of the study were analyzed [Table 1]. Due to the heterogeneity of the included reviews in this study, statistical analysis was not done.

Results

The systematic search identified 253 records. After removing duplicate records ($n = 21$) and those categorized as ineligible by automation tools ($n = 92$), 140 records were screened.

Table 1: Characteristics of the Pooled Data

S. No.	Author, Year	Sample Type	Total no. of Patients with Gastric Cancer with miRNA Expression	miRNA Studied	Results/Outcome
1.	Yao, *et al*., 2021[6]	Serum	40	has-miR320hashsa-miR-1260bhasnd hsa-miR-6515-5p	hsa-has320a, hsa-miR-1260b, and hsa-miR-6515-5p showed significantly high expression in the serum samples of gastric cancer patients.
2.	So, *et al*., 2021[7]	Serum	578	12-miR assay	Validated a 12-miRNA biomarker assay for serum, which may serve as a cost-effective risk assessment for gastric cancer.
3.	Wu, *et al*., 2015[8]	Serum, peripheral blood mononuclear cell (PBMC) isolation	50	miR-21	Significant elevation of miR-21 was evident in both serum and PBMC of GC patients compared to healthy controls
4.	Wu, *et al*., 2015[9]	Serum, peripheral blood mononuclear cell (PBMC) isolation	90	miR-421	The levels of miR-421 in both serum and PBMCs had higher sensitivity and specificity compared to CEA and CA-125 in GC diagnosis.
5.	Hou, *et al*., 2016[10]	Serum	150	miRNA-206	Serum miRNA-206 might not only serve as a novel diagnostic biomarker for GC, but also predict cancer recurrence and patient's prognosis.
6.	Tsujiura, *et al*., 2010[11]	Serum	69	*miR-17-5p*, *miR-21*, *miR-106a*, *miR-106b* and *let-7a*	Plasma levels of miRNAs (*miR-17-5p*, *miR-21*, *miR-106a*, *miR-106b*) were significantly higher in GC patients compared to controls, whereas *let-7a* levels were decreased in GC patients.
7.	Cuellar-Gomez, *et al*., 2021[12]	Plasma	80	miR-16, miR-21, miR-25, miR-26a, miR-92, miR-218, miR-223, and miR-451	Plasma levels of miR-21, miR-25, and miR-223 in plasma samples can serve as a potential diagnostic tool for GC.
8.	Zheng, *et al*., 2021[13]	Serum	168	Exosomal miR-590-5p	The decrease in the levels of exosomal miR-590-5p was correlated to the reduction of overall survival rate.
9.	Liu, *et al*., 2016[14]	Plasma	5	miR-940	Downregulation of plasma miR-940 may serve as a potential biomarker for detection of gastric cancer.
10.	Huang, *et al*., 2018[15]	Serum	24	miR-21-5p, miR-22-3p, and miR-29c-3p	Serum levels of miR-21 were significantly elevated in GC patients compared to the levels in healthy controls.
11.	Kaczor-Urbanowicz, *et al*., 2022[16]	Saliva	50	miR-140 and miR-301a	The proposed panel of biomarkers consisted of three mRNAs (SPINK7, PPL, and SEMA4B) and two miRNAs (miR-140-5p and miR-301a), which were significantly down-regulated in the GC group.
12.	Yosizawa, *et al*., 2012[17]	Saliva	15		15 salivary candidate miRNAs tested, 6 miRNAs were verified, and all significantly down-regulated.
13.	Li, *et al*., 2018[18]	Saliva	163	miR-140-5p, miR-374a,miR-454, miR-15b, miR-28-5p, and miR-301a	6 down-regulated miRNAs (miR-140-5p, miR-374a,miR-454, miR-15b, miR-28-5p, and miR-301a) were verified.
14.	Saliminejad, *et al*., 2022[19]	Plasma	97	miR-18a, 21, 25, 92a, 125b, and 221	A panel of circulating miR-18a/21/125b was suggested as a potential biomarker for the early detection of GC.

CA-125 = cancer antigen-125; CEA = carcinoembryonic antigen; GC = gastric carcinoma.

After considering the inclusion and exclusion criteria and based on eligibility and accessibility to the reports, 14 studies were finally included in the review. The detailed protocol of literature search using PRISMA guidelines is depicted using a flowchart. Fig. 1

From: Page MJ, McKenzie JE, Bossuyt PM, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. http://dx.doi.org/10.1136/bmj.n71 For more information, visit: http://www.prisma-statement.org/.

Risk of bias and applicability concerns were evaluated using the four domains of QUADAS-2: patient selection, index test, reference standard, procedure and timing [Figure 2]. Two to four questions with a ″yes,″ ″no,″ or ″unclear″ response make up each domain. Quality evaluation based on diagnostic accuracy studies conducted by Hou *et al.* (2016),[10] Jianhong

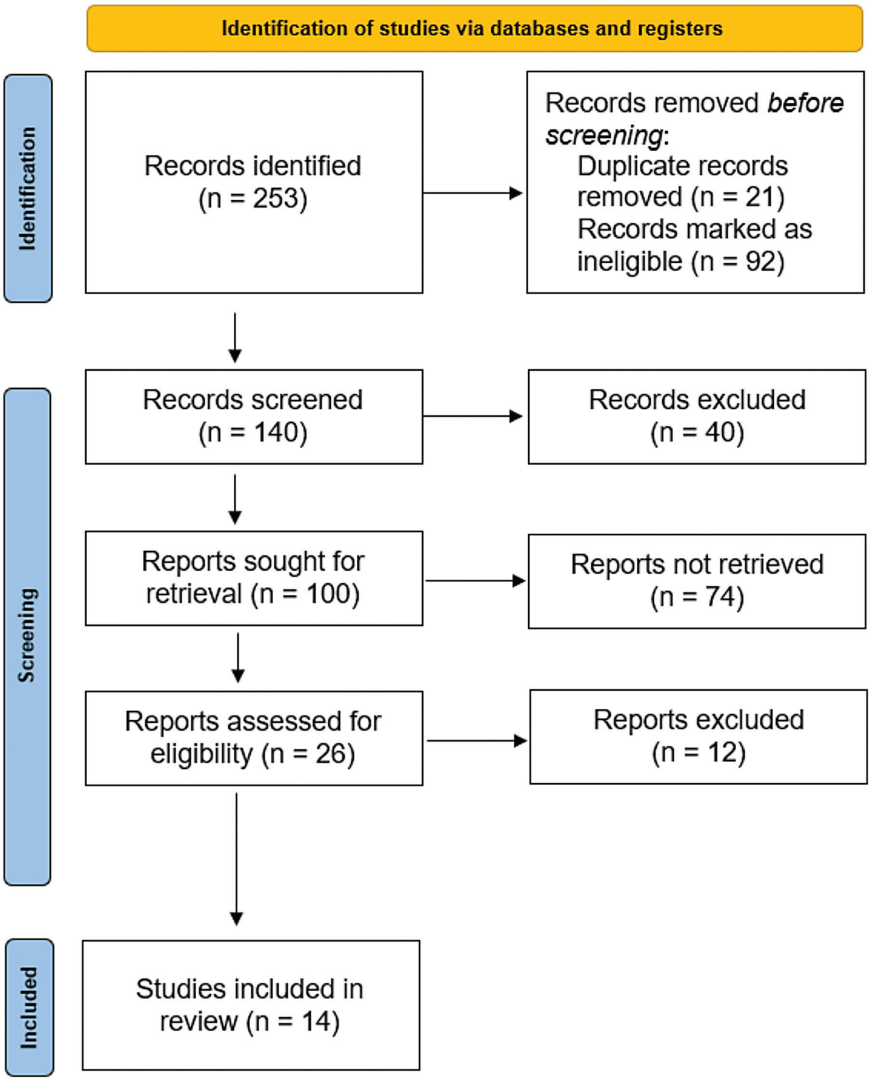

Figure 1: PRISMA flowchart depicting the included studies.

et al. (2015),[8] Kaczor-Urbanowicz *et al.* (2022),[16] Saliminejad *et al* (2022)[19], and So JBY *et al* (2021)[7] had the lowest risk of bias, according to a risk of bias chart created using Revman 5.4.1 software [Figure 3]. A serum 12-miRNA biomarker assay was validated by the low risk of bias research, and it may be a practical way to assess the likelihood of gastric cancer.

Discussion

Research on miRNAs as prospective biomarkers for cancer detection has gained momentum in recent past, as their expression has been dysregulated in many solid tumors. Aberrant expression of circulating miRNAs has been reported in biofluids like blood in patients with GC.[20] Considering the minute size of miRNAs, presence of numerous tissue sources for circulating miRNAs, and various physiological/pathological conditions that significantly alter miRNA concentrations, detection of alteration of miRNA levels in blood is a technically challenging task.[21] Research studies exploring the potential role of circulating miRNAs as GC biomarkers have yielded encouraging proof-of-concept findings, but are still mainly equivocal. This may be attributed to the small cohort sizes ($n = 6$–570) and the use of research-grade assays.[7] Lee JM *et al.* discovered salivary biomarkers that are unquestionably valid for GC detection.[22]

Based on a prospective clinical design, consistent with the PRoBE criteria for biomarker discovery, nine of the top 30 (30%) salivary mRNA candidates and four of the top 15 (27%) salivary miRNA biomarkers for GC were identified and verified.[17] The detected salivary biomarkers, both mRNA and miRNA, were validated in a cohort comprising of 100 GC participants and 100 non-GC controls, yielding a panel of 3 mRNAs (SPINK7, PPL, and SEMA4B) and 2 miRNAs (miR-140-5p and miR-301a), which showed the best performance with an AUC value of 0.81.

Few oncomiRs have been linked to oncogenesis in GC.[23] The oncomir miR-718 activates PI3K/Akt (phosphoinositide-3-

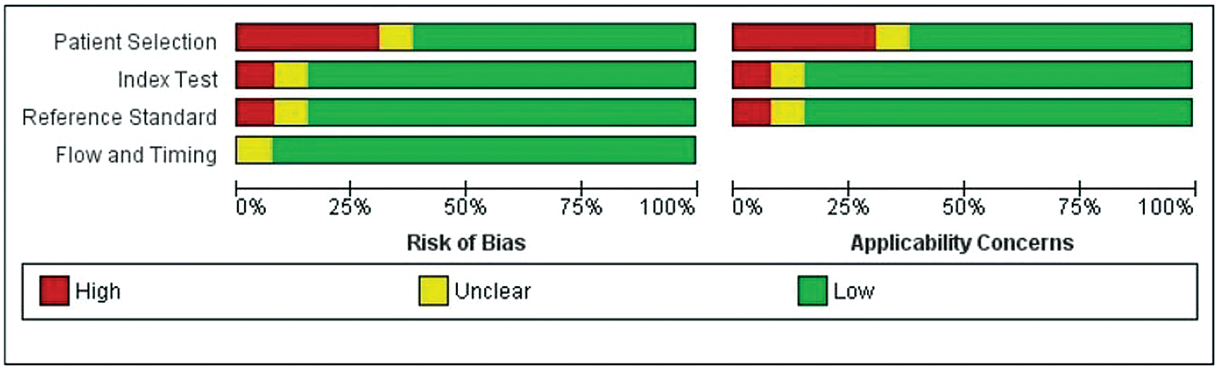

Figure 2: Risk of bias and applicability concerns graph: review authors' judgment about each domain presented as percentages across included studies.

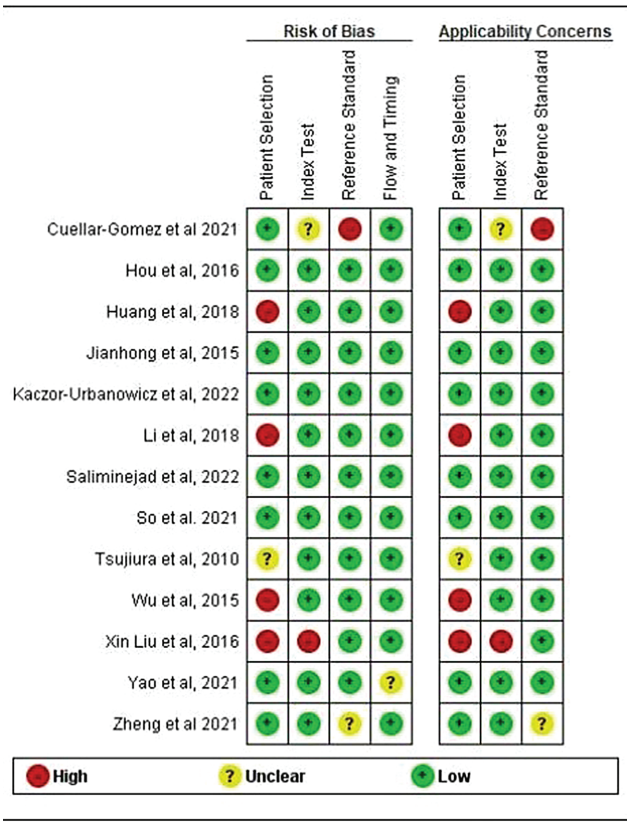

Figure 3: Risk of bias and applicability concerns summary: review authors' judgment about each domain presented as percentages across included studies.

kinase-protein kinase B/Akt) signaling, which directly downregulates PTEN (phosphatase and TENsin homolog deleted on chromosome 10) and promotes the proliferation and invasion in GC. PTEN expression is triggered by binding of KLF-2 (Krüppel-like Factor 2) to the PTEN promoter. The miRNA-32-5p downregulates KLF2 expression in order to lower PTEN and activate PI3K/AKT signaling and thereby promote the development of GC. PTEN levels are sharply decreased by miR-21, which causes an uptick in the phosphorylation of Akt at Thr308 and Ser473.[24] In conclusion, miR-21 targets the PTEN/Akt signaling pathway in human GC cells to promote cell migration and proliferation.[25]

MiR-15b-3p inhibits the expression of DYNLT1 (Dynein Light Chain Tctex – Type 1) and cleaved caspase – 9 in GC, which enhances migration, invasion, proliferation, and inhibits apoptosis.[26] Plasma levels of miR-21 and miR-222 are highly elevated in GC. High levels of SRC miR-99a5p expression are elicited in patients with specific histologic subtype of GC namely the Signet-ring cell carcinoma.[27]

The levels of miR-17 and miR-25 in GC tissues and cells are noticeably higher than those in normal tissues and cells. The miR-21 increases the chemoresistance of cisplatin (DDP) and curcumin as well as tumor development, migration, and invasion by inhibiting autophagy via the miR-21/PTEN/PI3K/Akt/mTOR pathway in GC cells.[28]

Majority of the published reports are focused on blood-based biomarkers for GC. So *et al.* performed a three-phase, multicenter study from Singapore and Korea in retrospective cohorts of 682 subjects and developed a 12-miR assay from serum specimens (miR-140, miR-183, miR-30e, miR-103a, miR-126, miR-93, miR-142, miR-21, miR-29c, miR-424, miR-181a, and miR-340). This study remains the most extensive evaluation of circulating miRNAs as biomarkers for GC diagnosis, both in terms of cohort size and technical stringency. The expression of 578 serum miRNAs in 682 cancer patients and control patients was quantified using a comprehensive biomarker detection panel that included numerous advancements to overcome the technical and biological challenges inherent in detecting circulating miRNA. Around 68 novel miRNAs associated with GC were discovered. Further evaluation using multivariant analysis yielded a 12-miRNA assay that discriminated between GC patients and matched high-risk controls with remarkable accuracy (AUC $>$ 0.93).[7]

The most technically challenging issue in this field of research is the difficulty in normalizing circulating miRNAs. Receiver Operator Curve (ROC) values generated as a result of multiple research studies in this field aid in gauging the diagnostic potential of miRNAs as

biomarkers in GC. For instance, Konishi *et al.* detected that miR-451 had a diagnostic accuracy of 0.96 with a computed sensitivity and specificity of 96% and 100%, respectively.[29] In their study of miR-940, Liu *et al.* obtained an ROC value of 0.96 with considerably lower sensitivity and specificity values of 81.2% and 98.6%, respectively.[14]

There is a significant amount of technical variation among the studies reviewed in the current systematic review, including different extraction kits, qPCR (quantitative Polymerase Chain Reaction) techniques, and most crucially, normalization. According to Komatsu *et al.*, both gastric cancer tissues ($n = 69$) and plasma expressed higher levels of miR-21, miR17-5p, miR-106a, and miR-106b.[30] In their work, Chen *et al.* explored the possibility whether certain plasma miRNAs could be linked to distant metastases. Their findings showed that patients with a distant metastasis had considerably elevated levels of miR-192. Similarly, plasma levels of miR-122 in the same patients were low. However, higher plasma levels of miR-122 were associated with higher survival rates.[31] Wu and coworkers discovered that, with an AUC, sensitivity, and specificity of 0.821, 95.5%, and 89.1%, respectively. There was overexpression of miR-421 in gastric cancer patient sera from 90 cases compared to sera from 90 controls. These numbers were comparatively higher than cancer antigen-125 and carcinoembryonic antigen used for the identification of stomach cancer.[32] The miR-421, a biomarker for circulating tumor cells from gastric cancer is highly expressed in mononuclear cells and may be utilized for early detection of gastric metastases.

In the study by Kaczor-Urbanowicz KE *et al.*, three mRNAs (SPINK7, PPL, and SEMA4B) and two miRNAs (miR-140-5p and miR-301a) were included in the constructed biomarker panel and were all found to be significantly down-regulated in the saliva of GC patients.[16] Further studies are needed to evaluate the diagnostic value of models in different ethnic populations.[15] This research emphasizes the need for creating and establishing population-specific salivary exRNA biomarkers for the detection of GC.[16] In the present review of 14 studies, Hou *et al.* (2016),[10] Jianhong *et al.* (2015),[8] Kaczor-Urbanowicz *et al.* (2022),[16] Saliminejad *et al.* (2022),[19] and So JBY *et al*. (2021)[7] had the least amount of bias. The study with a low risk of bias validated a serum 12-miRNA biomarker assay, which might be a useful tool for estimating the risk of gastric cancer. Hou *et al.* suggested that serum miRNA-206 can predict cancer recurrence and prognosis of patients, besides being a promising and novel diagnostic biomarker for GC.[10]

Numerous studies in the current review emphasized the enormous potential of miRNAs as markers for the diagnosis and prognosis of GC. Many of the included studies, however, suffered from one or more study design limitations, such as a small sample size, a lack of subgroup analysis, heterogeneous samples and study designs, the use of unreliable internal controls, and the failure to exclude participants with active or past cancer. Hence, a formal meta-analysis was not possible as most authors refrained from providing access to their molecular data.

Conclusion

This systematic review provides an insight into an array of diagnostic miRNAs reported from various body fluids and also outlines their potential role and application of these miRNAs as diagnostic biomarkers for the early detection of gastric cancer. The current review also provides a compilation of the reported panel of miRNAs in plasma, PBMNCs, serum, and saliva as diagnostic biomarkers in GC. The serum 12-miRNA biomarker assay, validated by a low risk of bias research is recommended to serve as a potential diagnostic tool for gastric cancer. However, the validation of these salivary, serum, and plasma miRNAs as a diagnostic biomarker for gastric cancer mandates further long-term follow-up studies in diverse ethnicity of populations.

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