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Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol

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ABSTRACT

Introduction The human stomach is a complex and diverse microbial ecosystem. Consecutive alternations of gastric microbiota occur in gastric carcinogenesis, while the changing pattern during this process remains controversial across studies. We aim to identify the changes in the diversity and composition of gastric mucosal microbiota in gastric tumorigenesis.

Methods and analysis We will search through PubMed, EMBASE and Cochrane databases, as well as conference proceedings and references of review articles for observational articles reporting either the relative abundance of bacteria at the phylum or genus level or at least one of the alpha diversity indexes respectively and clearly in both gastric cancer and non-cancer groups. Selection of studies and data extraction will be performed independently by two researchers. Disagreements will be resolved through discussion. Risk of bias will be assessed using the modified Newcastle-Ottawa Scale. Quantitative analyses will be performed using a random effects model, where the effect measurement will be expressed as the MD.

Ethics and dissemination Ethical approval for this systematic review is not required, as the study is based exclusively on published documents and will not include any individual data. Findings of this study are expected to be disseminated through peer-reviewed journals or conference proceedings.

PROSPERO registration number CRD42020206973.

Strengths and limitations of this study

- This systematic review will comprehensively identify changes in gastric mucosal microbiota diversity and composition during gastric carcinogenesis, an important but controversial clinical issue.
- Limited statistical power in published articles will be resolved through quantitative synthesis.
- Selection of articles, data extraction and evaluation of risk of bias will be performed by two researchers independently with disagreements resolved through discussion, minimising the potential personal biases.
- Given that the majority of studies concerning this issue are observational studies, we anticipate large heterogeneity across studies.

INTRODUCTION

The human gastrointestinal tract is a complex and diverse microbial ecosystem, which contains numerous microorganisms. Through interactions, microbes regulate a variety of physiological processes, as well as the occurrence and development of diseases.[1] Until the discovery of *Helicobacter pylori* in 1983, the stomach was thought to be a sterile environment, given its high gastric acid content and strict antimicrobial mechanisms. However, recent advances in high-throughput sequencing technology have helped uncover the unique and complex composition of gastric microbiota.[2]

Gastric cancer is the fifth most prevalent malignancy (1 033 701 new cases in 2018) and the third cause of cancer death (782 685 deaths in 2018) worldwide. The morbidity of gastric cancer continues to increase in recent years, particularly in regions with a high incidence of this disease, such as China and other Asian countries.[3 4] Correa's model of gastric carcinogenesis postulates that normal gastric mucosa will go through the progressive histological stages from non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia to eventually gastric cancer.[5] Numerous studies have implicated *H. pylori* infection in the development of gastric cancer.[6] However, only about 1% of patients with *H. pylori*-induced chronic gastritis ultimately develop cancer,[7] and the eradication of *H. pylori* does not completely prevent carcinogenesis.[8 9] On the other hand, increasing evidence has shifted the paradigm from *H. pylori* infection to the gastric microbiota dysbiosis, for the development of gastric cancer.[10 11]

Studies have demonstrated remarkable differences in gastric microbiota profile between non-cancer individuals and patients with gastric cancer, with microbial diversity changes and enrichments of certain bacteria while depletions of others.[10 12] Identifying the changes in gastric microbiota profile may

help in prevention, early diagnosis and management of gastric cancer. However, the gastric microbiota is diverse and dynamic and may be affected by several factors and differs geographically and ethnically.[13 14] Discrepancies were found across present studies, and the small sample sizes and heterogeneity of published studies have compromised the overall understanding of this issue. This underscores the need to perform a systematic review and meta-analysis to evaluate and to provide stronger evidence for the changes in the diversity and composition of gastric mucosal microbiota in gastric carcinogenesis.

Objectives

The purpose of this research protocol is to outline a systematic review and meta-analysis, which evaluates the changes in the diversity of gastric microbiota and the relative abundance of bacterial phyla and genera in the development of gastric cancer.

METHODS AND ANALYSIS

Our protocol adheres to the guideline of the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) statement.[15] Reporting items are detailed in PRISMA-P checklists (online supplemental appendix 1).

Inclusion criteria

Types of studies

This systematic review will include observational (cross-sectional, case–control, prospective and retrospective cohorts) human studies.

Study characteristics

Eligible studies should include both a group of patients with gastric cancer and a group of non-cancer patients whose diagnoses are confirmed by both clinical and histological evaluation. For histological evaluation, the gastric cancer should be confirmed as gastric adenocarcinoma. Histological diagnoses of non-cancer histological types including normal gastric mucosa, non-atrophic gastritis, atrophic gastritis and intestinal metaplasia shall comply with updated Sydney system.[16] Accordingly, normal gastric mucosa is defined as normal epithelium and glandular compartments with only individual scattered chronic inflammatory cells. Non-atrophic gastritis is defined as increased infiltration of chronic inflammatory cells without loss of gastric glands proper. Atrophic gastritis is defined as loss of gastric glands proper. Intestinal metaplasia is defined as the presence of goblet cells, absorptive cells and cells resembling colonocytes in the area of glands and mucosal epithelium. The diagnosis of intraepithelial neoplasia should be confirmed by revised Vienna classification system.[17] The *H. pylori* infection status should be determined on the basis of ^{13}C urea breath test or histological assessment. The source of samples will be limited to gastric biopsy samples (surgical or endoscopic). Studies based on faecal or oral samples will be excluded to avoid interference from intestinal and oral microbiota. In order to control methodological heterogeneity, we will only include studies using high-throughput sequencing technology.

Phenomenon of interest

Studies must report either the relative abundance of bacteria at the phylum or genus level or at least one of the alpha diversity indexes (the number of operational taxonomic units (OTUs), Shannon Index, Chao 1 Index, phylogenetic diversity and so on) in both gastric cancer and non-cancer groups.

Types of participants

We will only include participants who are 18 years or older. There are no further limitations on patient characteristics.

Literature searching strategy

We will search through PubMed, EMBASE and Cochrane databases for articles published up to 1 March 2021. The search terms shall include both free text and mesh terms to improve the search efficiency. Our search strategy in PubMed is ((“microbiome” OR “microbial” OR “microbiota” [MeSH Terms]) OR “microflora” OR “bacterial” OR “dysbiosis”) AND (“gastric” [MeSH Terms] OR “stomach” OR “upper digestive tract” OR “upper gastrointestinal tract”) AND ((“lesion” OR “cancer” [MeSH Terms] OR “neoplasia” OR “neoplasms” OR “malignancy” OR “tumor” OR “carcinoma” OR “adenocarcinoma” OR “premalignancy” OR “premalignant” OR “tumorigenesis” OR “carcinogenesis”) OR “intestinal metaplasia” OR “gastritis”) with the filter: “Humans”. The search strategy will be adapted for EMBASE and Cochrane databases. We will also search conference proceedings and the references of review articles for additional relevant studies. We will set no limitations on publication period or language.

Data collection and analysis

Selection of studies

Literature search results will be imported into a reference management software (EndNote), and duplicates will be removed. Two researchers (RJ and XZ) will preliminarily evaluate the eligibility of the articles by reading the title and abstract. The articles will then be divided into three categories: eligible, ineligible and pending. Ineligible articles will be eliminated. Two researchers will then independently read the full texts of eligible and pending articles, and articles meeting inclusion criteria will be recorded. Disagreements between the two researchers will be resolved by rechecking the article and discussion. Reasons for exclusions in each step will be recorded in EndNote library.

Data extraction and management

The data will be imported into Excel independently by two researchers (RJ and XZ). A senior researcher (YY) will double-check the extracted data. Disagreements will

be resolved through team discussion. We will retrieve the following information from each included study:

Information of the study

Publication (authors, year, journal title and format), study design (patient inclusion and exclusion criteria, source of samples, grouping and the sample size of each and sequencing technology) and bias control.

Patient characteristics

Demographics (age, sex, country or region, race/ethnicity and comorbidities), lesion location, clinical and histological diagnosis and *H. pylori* infection status.

Outcome data

The relative abundance of bacteria at the phylum or genus level and alpha diversity indexes, which include OTUs, Shannon Index, Chao 1 Index and phylogenetic diversity.

We will retrieve patient characteristics and outcome data in the cancer group and each histological type of non-cancer group, respectively. We will make full use of all available materials including published and unpublished articles or reports, online appendices and registration information. If required information is not clearly and completely recorded on the above sources, we will attempt to contact the corresponding author by email.

Risk of bias assessment

We will assess the risk of bias using a modified Newcastle-Ottawa Scale (NOS) (online supplemental appendix 2). NOS is a scoring system designed to evaluate the risk of bias in non-randomised studies, and we have incorporated adaptations based on the original version[18] with the intention of best evaluating our phenomenon of interest. The modified NOS additionally considers the following aspects: (a) subdivision of non-cancer lesions into normal gastric mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia according to histological evaluation; (b) clear exclusion criteria to prevent the impact of surgery or drugs on gastric microbiota; (c) sample size; (d) adjusting for *H. pylori* infection status and other demographic characteristics in analyses; and (e) description of detailed procedures and quality control of experiments. The assessment will be evaluated from three domains, selection, comparability and exposure (or outcome), and each study will be awarded with a maximum of 11 scores. The evaluation of the risk of bias will be performed independently by two researchers (RJ and XZ). Disagreements will be resolved through team discussion.

Data synthesis and statistical analysis

Basic characteristics and major outcomes of included studies will be tabulated first. The major outcomes refer to the changes in the diversity and composition of gastric microbiota (both statistically significant and non-significant) between gastric cancer and non-cancer groups. Only bacterial phyla or genera reported by five or more articles will be included in further meta-analysis.

The mean differences (MD) with 95% CI will be calculated as effect measurements. If data are reported as the median with IQR, we will convert them into the mean with SD through a recommended formula.[19] We will use the univariate analysis results unless multiple regression analyses are conducted. Moreover, we will extract the results from the regression model with the largest number of covariates if multiple models are used.

Additionally, we will compare the differences in alpha diversity indexes and relative abundance of bacterial phyla and genera between each non-cancer histological type (normal mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia) and the cancer group, respectively.

Considering the potential methodological, clinical and statistical heterogeneity across included observational studies, a random effects model will be used for data analysis. We will evaluate heterogeneity across studies using the Cochrane χ^2 and quantified with the I^2 statistics.[20] I^2 values of 25%, 50% and 75% will represent low, moderate and high heterogeneity, respectively.[21] Potential publication bias will be assessed by visual inspection of funnel plots, and the asymmetry of the funnel plot will be statistically examined using Egger's test.

We will conduct the following subgroup analyses to explore potential sources of heterogeneity: age, sex, race/ethnicity, comorbidities, country or region, *H. pylori* infection status, source of samples and sample size. Meta-regression will be performed to identify sources of heterogeneity across studies.

All analyses will be performed using Review Manager V.5.3.3 (Nordic Cochrane Centre, Copenhagen, Denmark). $P<0.05$ will be considered statistically significant.

Patient and public involvement

Patients or the public are not involved in the design, conduct, reporting or dissemination plans of our research.

Ethics and dissemination

This study is based on published data and will not include any human participants; thus, the ethical approval is not required. We have not published any data in a data repository as formal data collection has not started yet. Results of this study are expected to be published in peer-reviewed journals or conference abstracts.

DISCUSSION

Increasing evidence has indicated that consecutive alternations of gastric microbiota profile occur in gastric carcinogenesis. However, the changing pattern during this process remains largely unclear as the results differed across published articles.[10 12] Our systematic review and meta-analysis aim to identify the changes in the diversity and composition of gastric microbiota along the

normal to cancer cascade. Findings of this study have several potential clinical implications; first, to clarify the changing regularity of gastric microbiota profile in gastric carcinogenesis and, second, to identify specific microorganisms enriched in gastric tumorigenesis. The above implications may provide hints for exploring the involvement of gastric microorganisms in gastric mucosal immunity and its impact on the pathogenesis of gastric cancer,[22] as well as developing potential microbial therapy targets. Third, the detection of changes in gastric microbiota may be a diagnostic biomarker for gastric cancer. Despite the above clinical implications, our study has several limitations. Given the non-randomised nature of included observational studies, we anticipate large inter-study heterogeneity. Sources of heterogeneity should be further determined using subgroup analysis and meta-regression. Moreover, gastric mucosal microbiota, especially non-*H. pylori* bacteria, is a relatively young field, and the number of included studies is expected to be small. In addition, because we will only quantitatively analyse bacteria reported in at least five studies, certain important bacterial phyla and genera reported in lesser articles may be missed. Hence, with the continuous publication of articles in this field, the update of meta-analysis is warranted.

Contributors YY is the guarantor of this systematic review, initiated this research and designed the systematic review protocol. RJ, XZ and YZ contributed to the design and revision of the systematic review protocol. RJ, XZ and XC completed the pilot literature search and will conduct the formal selection of studies, data extraction, evaluation of risk of bias and quantitative synthesis. RJ, XZ and YY drafted the manuscript. All the authors will be involved in result interpretation. All the authors contributed to the review and revision of the manuscript and approved the publication.

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Disclaimer The sponsor has not been involved in study design, data collection, data analysis and result interpretation.

Competing interests None declared.

Patient consent for publication Not required.

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Present in review Y/N	Page and Line
ADMINISTRATIVE INFORMATION				
Title:				
Identification	1a	Identify the report as a protocol of a systematic review	Yes	Page 1 Line 3
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	No	/
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	Yes	Page 3 Line 1
Authors:				
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	Yes	Page 1 Line 4-17
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	Yes	Page 15 Line 16-23
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	No	/
Support:				
Sources	5a	Indicate sources of financial or other support for the review	Yes	Page 15 Line 25-26
Sponsor	5b	Provide name for the review funder and/or sponsor	Yes	Page 15 Line 25-26
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	Yes	Page 16 Line 1
INTRODUCTION				
Rationale	6	Describe the rationale for the review in the context of what is already known	Yes	Page 4 Line 3-Page 5 Line 11
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	Yes	Page 5 Line 13-16
METHODS				
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Yes	Page 6 Line 4-Page 7 Line 15

Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	Yes	Page 7 Line 17-Page 8 Line 8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Yes	Page 7 Line 17-Page 8 Line 8
Study records:				
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	Yes	Page 9 Line 1-4
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	Yes	Page 8 Line 11-20
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	Yes	Page 8 Line 11-20 Page 9 Line 14-19
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	Yes	Page 9 Line 5-13
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	Yes	Page 7 Line 7-11
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	Yes	Page 9 Line 21-Page 10 Line 14
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	Yes	Page 10 Line 17-21
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	Yes	Page 10 Line 22-Page 11 Line 14
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	Yes	Page 11 Line 17-20
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	Yes	Page 10 Line 17-21
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	Yes	Page 11 Line 14-16
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	No	/

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with both clinical and histological evaluations ✸
   b) yes, eg record linkage or based on self-reports
   c) no description
2) Representativeness of the cases
   a) consecutive or obviously representative series of cases ✸
   b) potential for selection biases or not stated
3) Selection of controls
   a) community controls ✸
   b) hospital controls
   c) no description
4) Definition of controls
   a) yes, with subdivision into normal mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia ✸
   b) yes, without further subdivision
   c) no description
5) Does the study have adequate exclusion criteria
   a) yes, have clear exclusion criteria, like history of surgery, history of taking antibiotics, prebiotics, probiotics, proton pump inhibitors (PPIs), chemotherapeutic drugs and any other drugs affecting gastric microbiota within the last month ✸
   b) no description
6) Study size
   a) ⩾50 participants in each group ✸
   b) <50 participants in each group

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for *H.pylori* infection status ✸
   b) study controls for age, sex, country or region, race/ethnicity ✸

Exposure

1) Ascertainment of the method
   a) detailed description of experimental procedures ✸

b) description of quality control✸
c) no description

2) Same method of ascertainment for cases and controls
a) yes ✸
b) no

3) Non-response rate
a) same rate for both groups ✸
b) non respondents described
c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
a) truly representative of the gastric cancer population✸
b) somewhat representative of the gastric cancer population ✸
c) selected group of users (eg, nurses, volunteers)
d) no description

2) Selection of the non-exposed cohort
a) drawn from the same community as the exposed cohort, with subdivision into normal mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia✸
b) drawn from the same community, without further subdivision
c) drawn from a different source
d) no description

3) Ascertainment of the method
a) detailed description of experimental procedures✸
b) description of quality control✸
c) no description

4) Demonstration that outcome of interest was not present at start of study
a) yes ✸
b) no

5) Does the study have adequate exclusion criteria
a) yes, have clear exclusion criteria, like history of surgery, history of taking antibiotics, prebiotics, probiotics, proton pump inhibitors (PPIs), chemotherapeutic drugs and any other drugs affecting gastric microbiota within the last month ✸
b) no description

6) Study size

a) ⩾50 participants in each group ✸

b) <50 participants in each group

Comparability

1) Comparability of cohorts on the basis of the design or analysis

a) study controls for *H.pylori* infection status ✸

b) study controls for age, sex, country or region, race/ethnicity ✸

Outcome

1) Study design

a) prospective ✸

b) retrospective

2) Assessment of outcome

a) independent blind assessment ✸

b) record linkage ✸

c) self-report

d) no description

3) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for ✸

b) subjects lost to follow up unlikely to introduce bias - small number lost - ⩾90 % (select an adequate %) follow up, or description provided of those lost) ✸

c) follow up rate < 90% (select an adequate %) and no description of those lost

d) no statement