BMJ Open

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

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To cite: Ji R, Zhao X, Cao X, *et al*. Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol. *BMJ Open* 2021;**11**:e045810. doi:10.1136/bmjopen-2020-045810

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-045810).

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Received 12 October 2020
Revised 04 February 2021
Accepted 15 February 2021

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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.

Funding This work was supported by Peking Union Medical College Hospital Youth Programme (grant number pumch201911356).

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.

Provenance and peer review Not commissioned; externally peer-reviewed.

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