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Intestinal barrier function in obese patients with or without metabolic syndrome: A systematic review protocol

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Intestinal barrier function in obese patients with or without metabolic syndrome: A systematic review protocol

Mariana Duarte Bona[1,2], Carlos Henrique de Medeiros Torres[1], Severina Carla Vieira Cunha Lima[1], Aldo Ângelo Moreira Lima[2], Bruna Leal Lima Maciel[1]

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Abstract

Introduction: Intestinal barrier function (IBF) is dependent on the structure and function of intestinal epithelial cells and paracellular pathway. The derangement of IBF can originate from conditions involving local and systemic chronic inflammation and metabolic diseases such as obesity and metabolic disorders. The aim of this paper was to describe a systematic review protocol with studies that determine the relationship between the intestinal barrier function, in obese patients with or without metabolic syndrome. **Methods and analysis:** This protocol is guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes Protocols (PRISMAP). The databases to be searched are PubMed, EMBASE, Scopus, ScienceDirect, and Web of Science. The systematic review will include original articles with adults and elderlies, obese with or without the diagnosis of metabolic syndrome, that address the intestinal barrier function in this population. Two independent reviewers will perform study selection, data extraction, and methodological quality assessment. Results corresponding to the analysis of intestinal barrier function between the studied groups will be described and will consider the difference in means and p values. Heterogeneity between study results will be assessed using a standard chi-squared test with a significance level of less than 0.05. The present protocol will assist in producing a systematic review that addresses if obesity or obesity associated with metabolic

syndrome alters intestinal barrier function. **Ethics and dissemination:** No ethical statement will be required. The results will be disseminated through a peer-reviewed publication and conference presentations.

Trial registration number: International Prospective Register for Systematic Reviews (PROSPERO) number CRD42020178658

Keywords: Intestinal barrier function, obesity, metabolic syndrome, systematic review

Strengths and limitations of this study:

- This study will focus in clinical research instead of the majority that focus in animal models researches;
- It will bring evidence of the most used *in vivo* tests to assess intestinal barrier function and integrity;
- In this study, obese with or without metabolic syndrome will be included;
- The scarcity of researches with elderlies and the methodological quality of studies may be the main limitations of the study.

1. Introduction

The incidence of obesity and metabolic syndrome has risen significantly worldwide over the last decades and reach epidemic proportions affecting all ages and socioeconomic groups.[1 2] Some evidence supports a causal pathway between diet, gut microbiota, intestinal barrier function and metabolic dysfunction.[3-5] Most of this knowledge is based on animal studies, where the link between alterations in the gut microbiota and, more recently, changes in intestinal barrier function was shown.[6]

The intestinal barrier is a complex multilayer system, consisting of an external physical barrier and an inner functional immunological barrier. The interaction of these two barriers enables the maintenance of equilibrated intestinal barrier function.[7] It prevents against loss of water and electrolytes and entry of antigens and microorganisms into the body while allowing the exchange of molecules between host and environment and the absorption of nutrients from the diet.[8]

Many factors can alter intestinal barrier function such as gut microbiota modifications, mucus layer alterations, and epithelial damage, resulting in translocation of luminal content to the inner layers of the intestinal epithelial cells.[9 10] Evidence obtained in animal models as well as in humans is accumulating supporting a role of

alterations of intestinal barrier function in many conditions, which include intestinal disorders such as malnutrition, diarrheal diseases, environmental enteric disease (EED), inflammatory bowel disease, irritable bowel syndrome, hepatic fibrosis, inflammation, sepsis and pancreatitis, but also obesity and metabolic syndrome.[11-14]

Intestinal barrier function and integrity can be measured in different ways. The techniques used for IBF and integrity assessment vary depending on the setting (*in vitro* versus *in vivo* measurements), the species (human or animals), the marker molecules used (ions, carbohydrates of different sizes, macromolecules and antigens, bacterial products and bacteria), and the compartments used for measurement of the marker molecules (peripheral blood, portal vein blood or urine).[15 16]

In vivo assessment of intestinal barrier absorption, damage and permeability in humans are currently possible by using intestinal barrier function biomarkers and assays. One of the most used assays is the lactulose:mannitol test, a quantitative non-invasive test that directly measures the ability of two non-metabolized sugar molecules, lactulose and mannitol, to permeate the intestinal mucosa.[17] Lactulose (L), a disaccharide, is absorbed through cell junctions or epithelial cell turnover or damage, while mannitol (M), a monosaccharide, is absorbed most across the epithelial cell membranes.[18] Once absorbed, these sugars are excreted unmetabolized in the urine. Elevated lactulose to mannitol ratio is an indicator of intestinal barrier dysfunction.[10]

Despite the test's immense potentials, its application in clinical research remains limited due to variations in the methodologies such as study population, sugar solution formulation and administration, urine collection time, and assay method to measure lactulose and mannitol between studies.[19] These variations restrict the clinical sensibility and accuracy of the lactulose:mannitol test, for example, the relationship between intestinal barrier function, integrity and inflammatory outcomes in diseases such as obesity and metabolic syndrome.

The aim of this paper is to describe a systematic review protocol with studies that determine the relationship between the intestinal barrier function, in obese patients with or without metabolic syndrome. In addition, the systematic review will evaluate methodologies used in the studies regarding intestinal barrier function biomarkers and assays methods. This review protocol will address if obesity or obesity associated with metabolic syndrome alters intestinal barrier function and integrity.

2. Methods

2.1 Protocol and registration

This protocol has been prepared according to the guidelines described in Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P).[20] A 27-item checklist was used to improve the quality of the systematic review data. The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on July 10, 2020 (CRD42020178658) and is available at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020178658.

2.2 Eligibility criteria

The search will be performed using journal articles that enhance methodological transparency. In this sense, the search will be elaborated and implemented before study selection, according to the PRISMA-P checklist as guidance. Additionally, journal articles that meet eligibility criteria using the Population, Intervention, Comparison, Outcome and Study design (PICOS) strategy will be included to ensure the systematic search of available literature.

2.2.1 Inclusion criteria

The review will include original articles with adults and elderlies, obese with or without the diagnosis of metabolic syndrome [according to modified National Cholesterol Education Program (NCEP) criteria is the presence of 3 or more of the following components: (1) waist circumference more than 90 cm in men or 80 cm in women; (2) triacylglycerols equal to or more than 150 mg/dL; (3) HDL-c less than 40mg/dL in men or 50 mg/dL in women; (4) blood pressure equal to or more than 130/85 mgHg and (5) fasting glucose between 100 and 125 mg/dL)][21], studies regarding the intestinal barrier function in this population, studies evaluating the intestinal epithelial cells integrity from obese patients with or without the diagnosis of metabolic syndrome.

2.2.2 Exclusion criteria

Review articles, case reports, comments, editorials, letters to the editor, theses, conference proceedings, studies with animals or cell models, studies with children, studies with adults and/or elderlies that have other metabolic diseases, studies that did not evaluate the intestinal barrier function.

2.3 Information sources and literature search

To identify the studies to be included in the systematic review, search strategies will be developed based on keywords indexed in the Medical Subject Headings (MeSH). The descriptors used will be related to intestinal barrier function, obesity, and metabolic syndrome, such as anthropometric data, biochemical analysis, intestinal permeability and integrity methodologies and assays. These descriptors will be accompanied by Boolean operators "AND" and "OR".

Two reviewers will independently conduct sensitive search for eligible systematic reviews through the electronic databases PubMed Database, Embase Database, Cochrane Library, Scopus, Web of Science (WOS) and Science Direct. Initial searches will test preliminary equations with the prospect of applying highly sensitive search strategies. Articles will be imported into Mendeley reference manager (1.19.4) and duplicates will be deleted. Initial screening of studies will be based on the information contained in their title, keywords and abstracts, following the eligibility criteria. When the reviewers disagree, the article will be re-evaluated and, if the disagreement persists, a third reviewer will make a final decision. Full-paper screening will be conducted by the same independent investigators. The references of the included articles will also be reviewed to identify those potentially eligible studies not found in the database search, considered as manual search.

2.4 Data extraction

For data extraction, two independent Microsoft Excel spreadsheets will be elaborated for two reviewers to summarize the data from the included studies. The following information will be extracted and entered in the spreadsheet: first author; year and language of publication; the country where the study was conducted; characteristics of the population (Metabolic Syndrome presence, age, gender, health conditions, total sample size, chronic diseases); methods to evaluate the intestinal barrier function; effects of obesity with or without metabolic syndrome in the intestinal barrier function; description of results and conclusions that are relevant to the overview; key findings; reported limitations.

2.5 Methodological quality assessment

Assessment of methodological quality and risk of bias in the studies with case-control design will be performed using Newcastle-Ottawa Scale, which includes eight items related to selection, comparison, and outcome.

2.6 Data analysis and synthesis

The systematic review will describe the relevant information of the included studies. Results corresponding to the analysis of intestinal barrier permeability between the studied groups will be described and will consider the difference in means and p values. Comparative analyses performed between the cases (obese with or without metabolic syndrome) and controls (healthy groups) will be presented. Heterogeneity between study results will be assessed using a standard chi-squared test with a significance level of 0.05.

3. Discussion

Obesity has become a global epidemic and is a substantial threat to patients and healthcare systems because of related morbidity and costs.[22] Metabolic and cardiovascular complications are a major obesity-associated burden, with critical roles for insulin resistance, type 2 diabetes and atherosclerosis.[23] Given the increasing prevalence of obesity worldwide, it is necessary to identify individuals with or without metabolic syndrome as a clinical priority.

Evidence has proposed the potential role of the gut microbiota as a pathogenic factor affecting host metabolic balance and disorders.[1] Gut microbiota seems to exert a great variety of functional properties impacting human physiology and pathology: modulation of host nutrition and energy harvest by the production of vitamins and fermentation of food components indigestible by the host; influence on intestinal epithelial homeostasis; intestinal barrier function; development of host immune system; protection against pathogens; drug metabolism.[6]

Animal models and some human studies are accumulating to support alterations of the intestinal barrier function in a vast array of conditions, which include obesity and metabolic syndrome.[24] Given the importance of the intestinal barrier function and integrity, understanding what can disrupt it and cause the loss of function and integrity are necessary. Even though no final conclusions exist, it is more evident that besides nutrients acting as down-regulators of tight junctions or as histone deacetylase (HDAC) inhibitors, also viral infections, toxins, hypoperfusion of the gut play a role. [25,26] Lifestyle factors such as living place, physical activity, dietary patterns and drug usage seem to play an important role as well, and they offer new approaches for improving gut barrier function.[26]

In this perspective, this systematic review will evaluate studies with obese patients with or without metabolic syndrome, focusing on the analysis of their intestinal barrier function. This review will also generate evidence for the use of lactulose:mannitol test for the diagnosis of *in vivo* intestinal barrier function and integrity.

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Contributors: MDB, CHMT, SCVCL and BLLM conceived the idea, planned and designed the study protocol. MDB and CHMT wrote the first draft; SCVCL and BLLM planned the data extraction and statistical analysis; AAML and BLLM provided critical insights. All authors have approved and contributed to the final written manuscript.

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Competing interests: None declared.

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Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	1
Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	7
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	1
Support			
Sources	#5a	Indicate sources of financial or other support for the review	7
Sponsor	#5b	Provide name for the review funder and / or sponsor	7
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	7
Introduction			
Rationale	#6	Describe the rationale for the review in the context of what is already known	2
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	3
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	4
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	4
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	4, 5
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	5
Study records - 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)	5
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for	5

		obtaining and confirming data from investigators	
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	4,5
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	4,5
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	5
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	5
Data synthesis	#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 I2, Kendall's τ)	5
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	5
Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	5
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	5
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	5

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The human protein zonulin is the main physiological modulator of tight junctions (TJs) in the intestinal epithelial layer that increases intestinal permeability in small intestine by inducing the opening of TJ and also participates in intestinal innate

immunity.[23] Circulating zonulin in serum is considered as a useful marker of intestinal barrier integrity and is measured using enzyme-linked immunosorbent assay. [24] In humans, it has been validated using lactulose/mannitol tests, being serum zonulin strongly correlated with the lactulose/mannitol ratio.[25]

Despite the tests immense potentials, application in clinical research remains limited due to variations in the methodologies such as study population, sugar solution formulation and administration, urine collection time, assay method and sensitivity.[26] These variations restrict the clinical sensibility and accuracy of the diagnostic tests of intestinal permeability, for example, the relationship between intestinal barrier function, integrity and inflammatory outcomes in diseases such as obesity and metabolic syndrome.

Animal models have shown that communication between the gut-adipose tissue and the gut-brain is essential for maintaining energy balance, and this communication is impaired during obesity and type 2 diabetes.[27] In this context, metabolic endotoxemia, characterized by an increase in lipopolysaccharides in plasma, was identified as one of the main factors that lead to the development of metabolic inflammation and insulin resistance. Increasing evidence supports that the intestinal microflora is responsible for the development of a low-grade inflammation that generates dysfunctions in the intestinal barrier, increases its permeability, and allows a consequent endotoxemia.[28]

Although these findings are well delineated for animal models, few studies in humans have been performed.[29] A study compared two groups of women with and without obesity, assessing intestinal permeability by urinary lactulose/mannitol ratio. Although both sugars' urinary excretions were higher in women with obesity, a statistically significant difference in the lactulose/mannitol ratio was not found between the studied groups. Nevertheless, a higher lactulose/mannitol ratio was associated with higher homeostatic model assessment (HOMA), insulin and LDL/HDL concentrations, and lower HDL concentrations.[30] Thus, the intestinal barrier function might be associated with obesity and metabolic syndrome.

This study aims to describe a systematic review protocol investigating if obesity with or without metabolic syndrome is associated with an altered intestinal barrier function. In addition, the systematic review will evaluate methodologies used in the studies regarding intestinal barrier function biomarkers and assays methods. This review protocol will address if obesity or obesity associated with metabolic syndrome alters intestinal barrier function and integrity.

2. Methods

2.1 Patient and public involvement

No patients involved.

2.2 Protocol and registration

This protocol has been prepared according to the guidelines described in Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P).[31] A 27-item checklist was used to improve the quality of the systematic review data. The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on July 10, 2020 (CRD42020178658) and is available at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020178658.

2.3 Eligibility criteria

Observational studies, published in scientific journals, will be included in the review. The guiding question in order to ensure the systematic search of available literature is '*'Is there alteration of intestinal barrier function in individuals with obesity with or without metabolic syndrome?''*. Thus, studies that have addressed as population individuals with obesity with or without metabolic syndrome assessing intestinal permeability as a variable will be included in the systematic review.

2.3.1 Inclusion criteria

The review will include original articles studying adults and elderlies with obesity with or without metabolic syndrome [according to modified National Cholesterol Education Program (NCEP) criteria is the presence of 3 or more of the following components: (1) waist circumference more than 90 cm in men or 80 cm in women; (2) triacylglycerols equal to or more than 150 mg/dL; (3) HDL-c less than 40mg/dL in men or 50 mg/dL in women; (4) blood pressure equal to or more than 130/85 mgHg and (5) fasting glucose between 100 and 125 mg/dL)][32], studies regarding the intestinal barrier function in this population and studies evaluating the intestinal epithelial cells integrity from individuals with obesity with or without metabolic syndrome.

All diagnostic test for intestinal barrier function will be considered for the systematic review (urinary measurement of orally-administrated sugar probe molecules and assays that use zonulin as a marker for intestinal permeability).

2.3.2 Exclusion criteria

Review articles, case reports, comments, editorials, letters to the editor, theses, conference proceedings, studies with animals or cell models, studies with children, studies with adults and/or elderlies that have other metabolic diseases, studies that did not evaluate the intestinal barrier function.

2.4 Information sources and literature search

The search will be elaborated and implemented according to the PRISMA-P checklist as guidance. Search strategies will be developed based on keywords indexed in the Medical Subject Headings (MeSH) to identify the studies to be included in the systematic review. The descriptors used will be related to intestinal barrier function, obesity, and metabolic syndrome, such as anthropometric data, biochemical analysis, intestinal permeability and integrity methodologies and assays. These descriptors will be accompanied by Boolean operators "AND" and "OR".

Two reviewers will independently conduct sensitive search for eligible studies through the electronic databases PubMed Database, Embase Database, Cochrane Library, Scopus, Web of Science (WOS) and Science Direct. In order to reflect the latest data, a search of the literature from the last 15 years (2006 to 2021) will be performed. Articles will be imported into Mendeley reference manager (1.19.4) and duplicates will be deleted. Initial screening of studies will be based on the information in their title, keywords and abstracts, following the eligibility criteria. When the reviewers disagree, the article will be re-evaluated and, if the disagreement persists, a third reviewer will make a final decision. Full-paper screening will be conducted by the same independent investigators. The references of the included articles will also be reviewed to identify those potentially eligible studies not found in the database search, considered as manual search.

2.5 Data extraction

For data extraction, two independent Microsoft Excel spreadsheets will be elaborated for two reviewers to summarize the data from the included studies. The following information will be extracted and entered in the spreadsheet: first author; year and language of publication; the country where the study was conducted; characteristics of the population (Metabolic Syndrome presence, age, gender, health conditions, total sample size, chronic diseases); methods to evaluate the intestinal barrier function; effects of obesity with or without metabolic syndrome in the intestinal barrier function; description of results and conclusions that are relevant to the overview; key findings; reported limitations.

2.6 Methodological quality assessment

Assessment of methodological quality and risk of bias in the studies with case-control design will be performed using the adapted Newcastle-Ottawa Scale[33], in which studies that receive at least five stars (maximum of eight) will be classified as good quality studies. Two independent reviewers will assess the methodological quality of eligible studies. These independent reviewers will score the selected studies, and a third reviewer will resolve any disagreement.

2.7 Data analysis and synthesis

The systematic review will describe the relevant information of the included studies. Key information on characteristics, methods, results and quality scores of included studies will be tabulated. Following this, a narrative synthesis will be conducted.

Firstly, in the narrative review, the number of studies to be included in the synthesis will be reported and characteristics of each study will be described as well the location, kind and study population. Secondly, the narrative synthesis will report and discuss the methods used to evaluate the intestinal permeability and the relevant data. Also, the quality of the methods used will be discussed based on the related and observed study limitations. Finally, the observation of altered intestinal barrier function in obesity with or without metabolic syndrome will be explored and similarities and differences of findings will be reported.

The best-evidence synthesis will be guaranteed, and the risk of bias due to selective publication will be controlled by following the steps described above and assessing the quality of the evidence. The GRADE framework will be used to assess the quality of evidence concerning the association between intestinal barrier function alteration in obesity with or without metabolic syndrome. GRADE ranks the evidence as high (when there is strong certainty that the association is close to the estimated); moderate (when there is moderate certainty in the estimated association); low (when certainty in association is limited); and very low (when certainty in the association estimate is very limited owing to a significant degree of uncertainty in the findings).[34]

3. Discussion

Obesity has become a global epidemic and is a substantial threat to patients and healthcare systems because of related morbidity and costs.[35] Metabolic and

cardiovascular complications are a major obesity-associated burden, with critical roles for insulin resistance, type 2 diabetes and atherosclerosis.[36] Given the increasing prevalence of obesity worldwide, it is necessary to identify individuals with or without metabolic syndrome as a clinical priority.

Evidence has proposed the potential role of the gut microbiota as a pathogenic factor affecting host metabolic balance and disorders.[1] Gut microbiota seems to exert a great variety of functional properties impacting human physiology and pathology: modulation of host nutrition and energy harvest by the production of vitamins and fermentation of food components indigestible by the host; influence on intestinal epithelial homeostasis; intestinal barrier function; development of host immune system; protection against pathogens; drug metabolism.[7]

Animal models and some human studies are accumulating to support alterations of the intestinal barrier function in a vast array of conditions, which include obesity and metabolic syndrome.[37] Given the importance of the intestinal barrier function and integrity, understanding what can disrupt it and cause the loss of function and integrity are necessary. Even though no final conclusions exist, it is more evident that besides nutrients acting as down-regulators of tight junctions or as histone deacetylase (HDAC) inhibitors, also viral infections, toxins, hypoperfusion of the gut play a role. [38 39] Lifestyle factors such as living place, physical activity, dietary patterns and drug usage seem to play an important role as well, and they offer new approaches for improving gut barrier function.[39]

In this perspective, this systematic review will address studies that evaluated individuals with obesity with or without metabolic syndrome, focusing on the analysis of their intestinal barrier function. This review will also generate evidence for the use of different tests for the diagnosis of *in vivo* intestinal barrier function and integrity.

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Contributors: MDB, CHMT, SCVCL and BLLM conceived the idea, planned and designed the study protocol. MDB and CHMT wrote the first draft; SCVCL and BLLM planned the data extraction and statistical analysis; AAML and BLLM provided critical insights. All authors have approved and contributed to the final written manuscript.

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Competing interests: None declared.

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Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title			
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	1

Registration			
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Authors			
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	8
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	1
Support			
Sources	#5a	Indicate sources of financial or other support for the review	8
Sponsor	#5b	Provide name for the review funder and / or sponsor	8
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	8
Introduction			

Rationale	#6	Describe the rationale for the review in the context of what is already known	2
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4
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	5
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	6
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	6
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6
Study records - 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)	6,7

Study records - 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	6
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	5
Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	
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	7
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesized	
Data synthesis	#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 I2, Kendall's τ)	
Data synthesis	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	

Data synthesis	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	7
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	7