BMJ Open

Metabolomics for prediction of hypertension in pregnancy: a systematic review and meta-analysis protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-040652
Article Type:	Protocol
Date Submitted by the Author:	19-May-2020
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Keywords:	Hypertension < CARDIOLOGY, Prenatal diagnosis < OBSTETRICS, EPIDEMIOLOGY

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SYSTEMATIC REVIEW PROTOCOL

Metabolomics for prediction of hypertension in pregnancy: a systematic review and meta-analysis protocol

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Abstract

Introduction: hypertension is a very important cause of maternal morbidity and mortality worldwide, despite efforts on prevention. The lack of a tool to provide effective and early prediction of hypertension for a high-risk group may contribute to improve maternal and fetal outcomes. Metabolomics has figured out as a promised technology to contribute to the improvement on hypertension in pregnancy prediction. **Methods and analysis**: our primary outcome is hypertensive disorders of pregnancy. A detailed systematic literature search will be performed in electronic databases, using controlled terms 'preeclampsia', 'hypertensive disorders', 'metabolomics' and 'prediction' (and their variations). Studies from the latest twenty years will be included, except case reports, reviews, cross-sectional studies, letter to editors, expert opinions, commentaries papers or non-human research. If possible, we will perform a meta-analysis. Two peer reviewers will independently perform the search and in cases of discordance a third reviewer will be consulted. **Ethics and dissemination:** the results of this review will present the current use and performance of metabolomics for predicting gestational hypertension. Such data could potentially guide future studies and interventions to improve existing prediction models.

Strengths and limitations of this study

- Electronic search will cover the most important current available scientific databases for health research;
- There will not be a language restriction;
- Considering the complexity of Metabolomics technology and its methods, there would be a limitation to perform a quantitative synthesis.

Key words

Preeclampsia, pregnancy, hypertension, hypertensive disorders, hypertensive syndromes, metabolomics, metabolome

Prospero register number: CRD42018097409

Introduction

Hypertensive disorders in pregnancy consist of a group of conditions including preeclampsia, gestational hypertension, preeclampsia superimposed to chronic hypertension, white coat hypertension, masked hypertension and transient hypertension [1] [2] and appear as the second cause of maternal death in the world according to a study performed by the World Health Organization between 2003 and 2009 [3]. Preeclampsia is the leading cause of maternal morbidity and mortality in Brazil and in several other low-and middle-income countries [4] [5]. Its prevalence can vary according to the set of analyses, but the number ranges from 2 to 10% of all pregnancies [4]. Every year, around 80 thousand women die because of preeclampsia and its complications [6], despite potential prevention implemented by low-dose aspirin [7]. This intervention can represent a reduction rate of around 50% in the incidence of the early-onset preeclampsia cases, which developed preeclampsia before 34 weeks of gestation [7] [8]. In this scenario, prediction of pregnant women under high-risk to develop preeclampsia is a key topic.

Some biomarkers have been proposed as earlier predictors (placental growth factor - PIGF, pregnancy-associated plasma protein A-PAPP-A) combined with clinical factors (pulsatility index of uterine arteries at Dopplervelocimetry exam, mean arterial blood pressure), showing different and sometimes conflicting detection rates [9-12]. These studies present limitations regarding the number of participants enrolled and heterogeneity to assess the prediction performance of those factors. Furthermore, the proposed prediction models from combining those factors outline better detection rates for early-onset preeclampsia cases compared to late-onset cases [13-15].

In the last decade, with the broad application of omics technologies, metabolomics has been pointed as a promising tool for the identification of early predictors for many health disturbances [16-18] and preeclampsia is one of them. Through metabolomics, it would be possible to identify metabolites involved in the final line of gene expression and a phenotypic signature in high resolution of the disease to be studied [19-21]. Studies have provided some insights about preeclampsia prediction through metabolites, belonging to different chemical classes and showing different performances [20-23]. In 2010, Kenny et al provided the initial knowledge on the topic, identifying 14 metabolites belonging to different chemical classes. When combined in an algorithm they showed a very good performance, with an Area under the Curve (AUC) of 0.94 in a discovery phase of the study and a detection rate of 77%, considering a false-positive rate of 10% [22]. It represents a very important tool option for prediction, especially with regard to cases of late-onset preeclampsia, which are the majority and the most difficult cases to predict [13-15]. Thus, in the sense of the inexistence of a systematic review protocol registered in this topic as well as a systematic review in progress or published, the main objective of this systematic review is to determine the accuracy of metabolomics for predicting hypertensive disorders of pregnancy.

Question formulation

In view of the social and economic implication of hypertensive disorders, their consequences to maternal and fetal lives worldwide and the lack of a useful screening test, in parallel to the increase of applicability of omics technologies, this systematic review will

be guided by this question: what is the performance of metabolomics for predicting gestational hypertensive disorders? It is in accordance with the PICO method [24] and associated with the search strategy provided a preliminary flow chart of studies as summarized in figure 1.

Methods and Analysis

Search Strategy

Electronic searches of literature will be carried out with these following databases: PubMed, EMBASE, Scopus, Web of Science, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE). We will include studies from the latest twenty years, considering that the vast majority of manuscripts on metabolomics are from this century. Our search strategy will combine terms with Boolean connectors related to the following categories: 1) hypertensive disorders, preeclampsia, pregnancy; 2) metabolomics, metabolome; and 3) screening, prediction. The Boolean connectors will be adapted according to the database used. We decided to use regular terms – not MESH or EMTREE terms – taking into account the number of databases consulted, in order to use always the same terms for all of them. In addition, we will search reference list of included articles, doing the backtracking of references. There will not be a language restriction. Before final publication, we will perform a new search in the databases in order to check if any study was published during the period of the systematic review elaboration. The databases exploration process and its results will follow the PRISMA Statement [25].

Study selection process

After searching all sources of databases cited above, all the citations will be exported into EndNote® software. Firstly, two reviewers (JM and DFBL) will independently assess titles and abstracts. Only papers considered potentially relevant according to the inclusion criteria will be retrieved for further consideration. Cases of divergence will be analyzed by a third reviewer (MLC) who will do the final decision. A fourth reviewer (JGC) will check all procedures before approving the data extraction.

Study inclusion criteria

Hypertensive disorders developed at any gestational age will be considered the domain studied. Previous other chronic conditions (diabetes, renal diseases, etc.) will be reported for stratification of analysis if the data allow for this. Original studies – including diagnostic studies - involving pregnant women are the inclusion criteria, and congenital malformation is the exclusion criteria.

Interventions/exposure

Prediction of hypertensive disorders through metabolomics technologies is the intervention to be studied. The biomarker analysis should have been performed on samples taken before the hypertensive disorder diagnosis.

Design

Our systematic review will include original studies (cohort or case control studies), including single or multiple pregnancies, as the studied population, and hypertensive disorders developed at any time of pregnancy, as the outcome of interest. We will exclude any studies that are: cross-sectional studies, case reports, editorials, letter to editors, commentaries,

expert opinions, any type of reviews, and experimental studies with animals, and when it is not possible to extract the data about the outcomes of interest.

Outcomes

We will include studies reporting outcomes of any hypertensive disorder developed during pregnancy. Our primary outcome is preeclampsia, defined as the onset of hypertension (a systolic blood pressure of 140 mmHg or more and/or a diastolic blood pressure of 90 mmHg or more) after 20 weeks of gestation, measured at least in two different occasions, combined with: (1) proteinuria (300 mg/day or at least 1g/L [1+] on dipstick testing or spot urine protein/creatinine>30mg/mmol [0.3mg/mg]) or (2) systemic complications or (3) uteroplacental dysfunction (fetal growth restriction) [1]. By systemic complications, we will consider:

- ✓ Hematological complications (thrombocytopenia- platelet count below 150,000/dL, disseminated intravascular coagulation, hemolysis);
- ✓ Hepatic dysfunction (elevated transaminases – at least twice upper limit of normal +- right upper quadrant or epigastric abdominal pain);
- ✓ Neurological dysfunction (examples include eclampsia, altered mental status, blindness, stroke or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata;
- ✓ Renal dysfunction (creatinine > 1.2mg/dL);

Secondary outcomes include:

- ✓ Early-onset preeclampsia: when occurs before or at 33 weeks of gestation [26];
- ✓ Late-onset preeclampsia: when occurs at or after 34 weeks of gestation [26].

- ✓ Gestational hypertension: de novo development of high blood pressure after 20 weeks of gestation (a systolic blood pressure of 140 mmHg or more and/or a diastolic blood pressure of 90 mmHg or more), without any of the abnormalities that define preeclampsia as discussed above [1]
- ✓ White coat hypertension: it is demonstrated when a normal blood pressure is registered during 24 hours ambulatory monitoring in the first half of pregnancy [1]
- ✓ Preeclampsia superimposed on chronic hypertension: in a patient with high blood pressure predating the pregnancy, it is registered the occurrence of preeclampsia [1]
- ✓ Masked hypertension: is characterized by blood pressure that is normal at office or clinic but elevated at other times, most typically diagnosed by 24 hours ambulatory blood pressure monitoring [2]
- ✓ Transient gestational hypertension: is hypertension that arises in the second or third trimester. The hypertension is detected in the clinic but then settles with repeated blood pressure readings [2]

Data extraction

Data will be extracted through a standardized data compilation form in duplicate to avoid errors. The variables of interest from each included study are: authors, country, year of publication, study design, number of participants, preeclampsia prevalence, gestational age of recruitment, biological samples utilized, laboratory methods, metabolomics technology applied and metabolites. The metabolites will be matched with the Human Metabolome Database (HMDB) in order to check their biological function and chemical subclass. Missing

data will be requested from study authors. Pairs of data-extraction forms will be checked for discrepancies.

Quality Appraisal

The same two reviewers (JM and DFBL) who judged eligibility of papers will independently assess the risk of bias in included studies, but this time rating the methodological quality of the primary research. A third reviewer (MLC) will solve divergences when needed. Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) is the standard scale to be applied to access internal validity [27]. This tool is composed by four domains: patient selection, index test (metabolomics technique), reference standard (arterial blood pressure) and flow and timing of patient inclusion and follow up. Each domain is assessed in terms of risk of bias and the first three are assessed in terms of concerns regarding applicability. For each domain, every study will be labelled as "low", "high" or "unclear" risk of bias.

Funnel plots and sensitivity and cumulative analyses will be applied for detection of temporal trends and publication bias.

Strategy for data synthesis

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), a flow diagram will be drawn [25]. Tables will show data regarding studies characteristics and risk of bias assessment for included and excluded studies. Narrative data will be analyzed and structured according to the outcomes: preeclampsia, gestational hypertension, transient gestational hypertension, white coat hypertension, masked hypertension. If possible, we are going to perform a subgroup analysis according to the metabolomics methods applied: gas or liquid chromatography, coupled with mass

spectrometry, or proton nuclear magnetic resonance. We also intend to perform a sensitivity analysis on the basis of early and late preeclampsia cases if sufficient studies will be found.

A meta-analysis will be performed (hierarchical summary receiver characteristic operating curve, HSROC) and accuracy measures will be calculated depending on data availability. Heterogeneity will also be assessed, through I-square test, Hotelling's T-squared test and Cochran's Q test.

Ethics and dissemination

Prediction of hypertensive disorders has been studied over the years with specific challenges. Among nulliparous for example, there is no history of previous events and a previous history of preeclampsia, is considered the most consistent predictive risk factor [28]. Another challenge to overcome is regarding to late-onset preeclampsia cases, which represent the majority of them. As cited above, the algorithms composed by biochemical and clinical factors showed better results with early onset cases of preeclampsia [13-14].

Metabolomics is a very complex technology and it has emerged as a possibility for prediction of adverse pregnancy outcomes [29-31]. The techniques employed are nuclear magnetic resonance spectroscopy, gas or liquid chromatography-mass spectrometry, Fourier transform infrared spectrometry and capillary electrophoresis [31]. Because of this complexity, results may be different concerning to the metabolites found. Consequently, generalizing results is also a challenge to overcome. This systematic review will contribute

to optimize the knowledge about the metabolites found in the studies and perhaps classify them according to HMDB, enabling quality translational research.

In addition, this systematic review will contribute to establish the current state of knowledge concerning the capacity of metabolomics to predict the occurrence of preeclampsia. Taking into account that this outcome involves relevant consequences for maternal and neonatal lives, the development of a tool that would predict preeclampsia is essential. Furthermore, the results of this systematic review could be used to guide future studies in this field. Once published, this systematic review will be free available in an open access scientific journal.

Patient and Public Involvement

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

List of abbreviations

PlGF- placental growth factor

PAPP-A- pregnancy-associated plasma protein A

AUC- area under the curve

LILACS- Latin America and Caribbean Health Sciences Literature

Scielo- Scientific Electronic Library Online

HTA- Health Technology Assessment

DARE- Database of Abstracts of Reviews of Effects

HMDB- Human Metabolome Database

QUADAS-2- Quality Assessment of Diagnostic Accuracy Studies

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analysis

HSROC- Hierarchical summary receiver characteristic operating curve

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Acknowledgements

This is a modified version of the article that was part of the PhD thesis of Jussara de Souza Mayrink Novais presented to the Postgraduate Program on Obstetrics and Gynecology from the School of Medical Sciences of the University of Campinas, Brazil, under the tutorial of Jose Guilherme Cecatti and Maria Laura Costa on December 13th 2018.

Authors' contributions

JM worked out the protocol, developed searches and data management, will participate in selection, inclusion, quality assessment and data extraction of papers. DFBL helped working out the protocol and will participate in selection, inclusion, quality assessment and data extraction as well. MLC and JGC helped working out the protocol, and MLC will solve any disagreement concerning to the selected papers. JGC will supervise all the development of the systematic review. All authors read and approved this final manuscript.

Funding

This study is a sub product of the study "Preterm SAMBA" which was jointly financed by the Brazilian CNPq (CNPq, Grant 401636/2013-5) and the Bill and Melinda Gates Foundation (Grant OPP1107597).

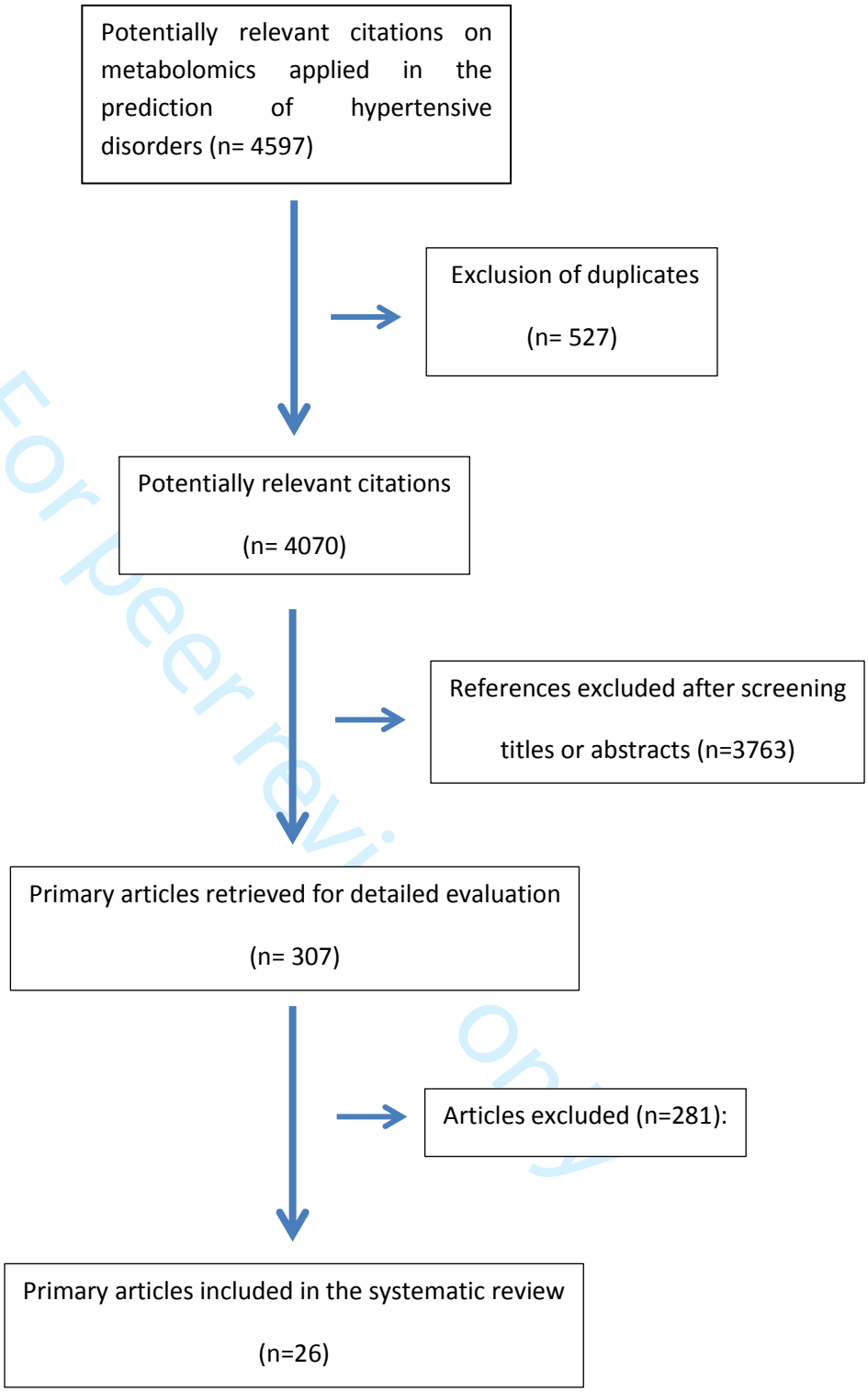
Competing interests

The author(s) declare that they have not competing interests.

Word count: 1973 words

Figure Legend

Figure 1. Flow chart of studies identified to be included in the systematic review.



PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - **Moher D, Stewart L & Shekelle P:** Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 **5**:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	x☐	☐	3
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	x☐	
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x☐	☐	52
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x☐	☐	5,6,7,8
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x☐	☐	347-353
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	☐	x☐	
Support					
Sources	5a	Indicate sources of financial or other support for the review	x☐	☐	344-345
Sponsor	5b	Provide name for the review funder and/or sponsor	x☐	☐	344-345
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	x☐	
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	x☐	☐	54-91

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	x☐	☐	94-99
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x☐	☐	107-109; 128-132; 140-143
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x☐	☐	104-107
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	x☐	☐	109-114
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x☐	☐	121-122
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x☐	☐	122-126
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x☐	☐	184-191
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☐	x☐	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x☐	☐	145-180
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	x☐	☐	193-204
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	x☐	
	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 (e.g., I^2, Kendall's tau)	☐	x☐	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	x☐	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x☐	☐	206-219

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	x☐	
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	x☐	

BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-040652.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Nov-2020
Complete List of Authors:	Mayrink, Jussara; Universidade Estadual de Campinas Faculdade de Ciencias Medicas Leite, Debora; State University of Campinas, Department of Tocogynecology; Universidade Federal de Pernambuco, Costa, Maria Laura Cecatti, Jose; State University of Campinas, Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Epidemiology
Keywords:	Hypertension < CARDIOLOGY, Prenatal diagnosis < OBSTETRICS, EPIDEMIOLOGY

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SYSTEMATIC REVIEW PROTOCOL

Metabolomics for prediction of hypertension in pregnancy: a systematic review and meta-analysis protocol

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Abstract

Introduction: hypertension is a very important cause of maternal morbidity and mortality worldwide, despite efforts on prevention. The lack of a tool to provide effective and early prediction of hypertension for a high-risk group may contribute to improving maternal and fetal outcomes. Metabolomics has figured out as a promised technology to contribute to the improvement of hypertension in pregnancy prediction. **Methods and analysis**: our primary outcome is hypertensive disorders of pregnancy. A detailed systematic literature search will be performed in electronic databases PubMed, EMBASE, Scopus, Web of Science, Latin America and Caribbean Health Sciences Literature, Scientific Electronic Library Online, Health Technology Assessment, and Database of Abstracts of Reviews of Effects using controlled terms 'preeclampsia', 'hypertensive disorders', 'metabolomics' and 'prediction' (and their variations). Studies from the latest twenty years will be included, except case reports, reviews, cross-sectional studies, letter to editors, expert opinions, commentaries papers or non-human research. If possible, we will perform a meta-analysis. Two peer reviewers will independently perform the search and in cases of discordance, a third reviewer will be consulted. **Ethics and dissemination:** as a systematic review, ethics approval is not required. The results of this review will present the current use and performance of metabolomics for predicting gestational hypertension. Such data could potentially guide future studies and interventions to improve existing prediction models.

Strengths and limitations of this study

- Electronic search will cover the most important current available scientific databases for health research;
- There will not be a language restriction;
- Considering the complexity of Metabolomics technology and its methods, there would be a limitation to perform a quantitative synthesis.

Keywords

Preeclampsia, pregnancy, hypertension, hypertensive disorders, hypertensive syndromes, metabolomics, metabolome

Prospero register number: CRD42018097409

Introduction

Hypertensive disorders in pregnancy consist of a group of conditions including preeclampsia, gestational hypertension, preeclampsia superimposed to chronic hypertension, white coat hypertension, masked hypertension and transient hypertension [1] [2] and appear as the second cause of maternal death in the world according to a study performed by the World Health Organization between 2003 and 2009 [3]. Preeclampsia is the leading cause of maternal morbidity and mortality in Brazil and several other low-and middle-income countries [4 5]. Its prevalence can vary according to the set of analyses, but the number ranges from 2 to 10% of all pregnancies [4]. Every year, around 70 thousand women die because of preeclampsia and its complications [3], despite potential prevention implemented by low-dose aspirin [6,7]. This intervention can represent a reduction rate of around 50% in the incidence of the early-onset preeclampsia cases, which developed preeclampsia before 34 weeks of gestation [7 8]. In this scenario, the prediction of pregnant women under high-risk to develop preeclampsia is a key topic.

Some biomarkers have been proposed as earlier predictors (placental growth factor - PIGF, pregnancy-associated plasma protein A-PAPP-A) combined with clinical factors (pulsatility index of uterine arteries at Dopplervelocimetry exam, mean arterial blood pressure) in models with different detection and false-positive rates [9-12]. These studies present limitations regarding the number of participants enrolled and heterogeneity to

assess the prediction performance of those factors. Furthermore, the proposed prediction models from combining those factors outline better detection rates for early-onset preeclampsia cases compared to late-onset cases [13-15].

In the last decade, with the broad application of omics technologies, metabolomics has been pointed as a promising tool for the identification of early predictors for many health disturbances [16-18] and preeclampsia is one of them. Through metabolomics, it would be possible to identify metabolites involved in the final line of gene expression and a phenotypic signature in high resolution of the disease to be studied [19-21]. Studies have provided some insights about preeclampsia prediction through metabolites, belonging to different chemical classes and showing different performances [20-23]. In 2010, Kenny et al provided the initial knowledge on the topic, identifying 14 metabolites belonging to different chemical classes. When combined in an algorithm they showed a very good performance, with an Area under the Curve (AUC) of 0.94 in a discovery phase of the study and a detection rate of 77%, considering a false-positive rate of 10% [22]. It represents a very important tool option for prediction, especially concerning cases of late-onset preeclampsia, which are the majority and the most difficult cases to predict [13-15]. Thus, in the sense of the inexistence of a systematic review protocol registered in this topic as well as a systematic review in progress or published, the main objective of this systematic review is to determine the accuracy of metabolomics for predicting hypertensive disorders of pregnancy.

Question formulation

Because of the social and economic implication of hypertensive disorders, their consequences to maternal and fetal lives worldwide and the lack of a useful screening test, in parallel to the increase of applicability of omics technologies, this systematic review will be guided by this question: what is the performance of metabolomics for predicting gestational hypertensive disorders? It is following the PICO method [24] and associated with the search strategy provided a preliminary flow chart of studies as summarized in figure 1.

Methods and Analysis

Search Strategy

Electronic searches of literature will be carried out with these following databases: PubMed, EMBASE, Scopus, Web of Science, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE). We will include studies from the latest twenty years, considering that the vast majority of manuscripts on metabolomics are from this century. Our search strategy will combine terms with Boolean connectors related to the following categories: 1) hypertensive disorders, preeclampsia, pregnancy; 2) metabolomics, metabolome; and 3) screening, prediction. The Boolean connectors will be adapted according to the database used. We decided to use regular terms – not MESH or EMTREE terms – taking into account the number of databases consulted, to use always the same terms for all of them. Also, we will search reference list of included articles, doing the backtracking of references. There will not be a language restriction. Before final publication, we will perform a new search in the databases to check if any study was published during

the period of the systematic review elaboration. The databases exploration process and its results will follow the PRISMA Statement [25].

Study selection process

After searching all sources of databases cited above, all the citations will be exported into EndNote® software. Firstly, two reviewers (JM and DFBL) will independently assess titles and abstracts. Only papers considered potentially relevant according to the inclusion criteria will be retrieved for further consideration. Cases of divergence will be analyzed by a third reviewer (MLC) who will do the final decision. A fourth reviewer (JGC) will check all procedures before approving the data extraction.

Study inclusion criteria

Hypertensive disorders developed at any gestational age will be considered the domain studied. Previous other chronic conditions (diabetes, renal diseases, etc.) will be reported for stratification of analysis if the data allow for this. Original studies – including diagnostic studies - involving pregnant women are the inclusion criteria, and congenital malformation is the exclusion criteria.

Interventions/exposure

Prediction of hypertensive disorders through metabolomics technologies is the intervention to be studied. The biomarker analysis should have been performed on samples taken before the hypertensive disorder diagnosis.

Design

Our systematic review will include original studies (cohort or case-control studies), including single or multiple pregnancies, as the studied population, and hypertensive disorders

developed at any time of pregnancy, as the outcome of interest. We will exclude any studies that are: cross-sectional studies, case reports, editorials, letter to editors, commentaries, expert opinions, any type of reviews, and experimental studies with animals, and when it is not possible to extract the data about the outcomes of interest.

Outcomes

We will include studies reporting outcomes of any hypertensive disorder developed during pregnancy. Our primary outcome is preeclampsia, defined as the onset of hypertension (systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure of 90 mmHg or more) after 20 weeks of gestation, measured at least in two different occasions, combined with (1) proteinuria (300 mg/day or at least 1g/L [1+] on dipstick testing or spot urine protein/creatinine>30mg/mmol [0.3mg/mg]) or (2) systemic complications or (3) uteroplacental dysfunction (fetal growth restriction) [1]. By systemic complications, we will consider:

- ✓ Hematological complications (thrombocytopenia- platelet count below 150,000/dL, disseminated intravascular coagulation, hemolysis);
- ✓ Hepatic dysfunction (elevated transaminases – at least twice upper limit of normal +- right upper quadrant or epigastric abdominal pain);
- ✓ Neurological dysfunction (examples include eclampsia, altered mental status, blindness, stroke or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata;
- ✓ Renal dysfunction (creatinine > 1.2mg/dL);

Secondary outcomes include:

- ✓ Early-onset preeclampsia: when occurs before or at 33 weeks of gestation [26];
- ✓ Late-onset preeclampsia: when occurs at or after 34 weeks of gestation [26].
- ✓ Gestational hypertension: de novo development of high blood pressure after 20 weeks of gestation (systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure of 90 mmHg or more), without any of the abnormalities that define preeclampsia as discussed above [1]
- ✓ Whitecoat hypertension: it is demonstrated when normal blood pressure is registered during 24 hours ambulatory monitoring in the first half of pregnancy [1]
- ✓ Preeclampsia superimposed on chronic hypertension: in a patient with high blood pressure predating the pregnancy, it is registered the occurrence of preeclampsia [1]
- ✓ Masked hypertension: is characterized by blood pressure that is normal at office or clinic but elevated at other times, most typically diagnosed by 24 hours ambulatory blood pressure monitoring [2]
- ✓ Transient gestational hypertension is hypertension that arises in the second or third trimester. The hypertension is detected in the clinic but then settles with repeated blood pressure readings [2]

Data extraction

Data will be extracted through a standardized data compilation form in duplicate to avoid errors. The variables of interest from each included study are: authors, country, year of publication, study design, number of participants, preeclampsia prevalence, gestational age of recruitment, biological samples utilized, laboratory methods, metabolomics technology

applied and metabolites. The metabolites will be matched with the Human Metabolome Database (HMDB) to check their biological function and chemical subclass. Missing data will be requested from study authors. Pairs of data-extraction forms will be checked for discrepancies.

Quality Appraisal

The same two reviewers (JM and DFBL) who judged eligibility of papers will independently assess the risk of bias in included studies, but this time rating the methodological quality of the primary research. A third reviewer (MLC) will solve divergences when needed. Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) is the standard scale to be applied to access internal validity [27]. This tool is composed of four domains: patient selection, index test (metabolomics technique), reference standard (arterial blood pressure) and flow and timing of patient inclusion and follow up. Each domain is assessed in terms of risk of bias and the first three are assessed in terms of concerns regarding applicability. For each domain, every study will be labelled as “low”, “high” or “unclear” risk of bias.

Funnel plots and sensitivity and cumulative analyses will be applied for the detection of temporal trends and publication bias.

Strategy for data synthesis

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), a flow diagram will be drawn [25]. Tables will show data regarding studies characteristics and risk of bias assessment for included and excluded studies. Narrative data will be analyzed and structured according to the outcomes: preeclampsia, gestational hypertension, transient gestational hypertension, white coat hypertension, masked

hypertension. If possible, we are going to perform subgroup analysis according to the metabolomics methods applied: gas or liquid chromatography, coupled with mass spectrometry, or proton nuclear magnetic resonance, and based on ethnic group and the severity of the hypertensive disease. We also intend to perform a sensitivity analysis based on early and late preeclampsia cases if sufficient studies will be found.

A meta-analysis will be performed (hierarchical summary receiver characteristic operating curve, HSROC) and accuracy measures will be calculated depending on data availability. If a meta-analysis will be possible, considering the limitations imposed by data heterogeneity and drawings of the vast majority of studies, we intend to use RevMan software. Taking into account that the studies involve the frequency of metabolites and occurrence of preeclampsia, we are going to use a fixed-effect model or random- effect model, depending on the heterogeneity found. Heterogeneity will also be assessed, through the I-square test, Hotelling's T-squared test and Cochran's Q test.

Ethics and dissemination

Prediction of hypertensive disorders has been studied over the years with specific challenges. Among nulliparous for example, there is no history of previous events and a previous history of preeclampsia, is considered the most consistent predictive risk factor [28]. Another challenge to overcome is regarding late-onset preeclampsia cases, which represent the majority of them. As cited above, the algorithms composed by biochemical and clinical factors showed better results with early-onset cases of preeclampsia [13-14].

Metabolomics is a very complex technology and it has emerged as a possibility for prediction of adverse pregnancy outcomes [29-31]. The techniques employed are nuclear magnetic resonance spectroscopy, gas or liquid chromatography-mass spectrometry, Fourier transforms infrared spectrometry and capillary electrophoresis [31]. Because of this complexity, results may be different concerning the metabolites found. Consequently, generalizing results is also a challenge to overcome. This systematic review will contribute to optimize the knowledge about the metabolites found in the studies and perhaps classify them according to HMDB, enabling quality translational research.

Besides, this systematic review will contribute to establishing the current state of knowledge concerning the capacity of metabolomics to predict the occurrence of preeclampsia. Taking into account that this outcome involves relevant consequences for maternal and neonatal lives, the development of a tool that would predict preeclampsia is essential. Furthermore, the results of this systematic review could be used to guide future studies in this field. Once published, this systematic review will be freely available in an open-access scientific journal.

Patient and Public Involvement

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

List of abbreviations

PlGF- placental growth factor

PAPP-A- pregnancy-associated plasma protein A

AUC- area under the curve

LILACS- Latin America and Caribbean Health Sciences Literature

Scielo- Scientific Electronic Library Online

HTA- Health Technology Assessment

DARE- Database of Abstracts of Reviews of Effects

HMDB- Human Metabolome Database

QUADAS-2- Quality Assessment of Diagnostic Accuracy Studies

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analysis

HSROC- Hierarchical summary receiver characteristic operating curve

Acknowledgements

This is a modified version of the article that was part of the PhD thesis of Jussara de Souza Mayrink Novais presented to the Postgraduate Program on Obstetrics and Gynecology from the School of Medical Sciences of the University of Campinas, Brazil, under the tutorial of Jose Guilherme Cecatti and Maria Laura Costa on December 13th 2018.

Authors' contributions

JM worked out the protocol, developed searches and data management, will participate in the selection, inclusion, quality assessment and data extraction of papers. DFBL helped working out the protocol and will participate in selection, inclusion, quality assessment and data extraction as well. MLC and JGC helped working out the protocol, and MLC will solve any disagreement concerning the selected papers. JGC will supervise all the development of the systematic review. All authors read and approved this final manuscript.

Funding

This study is a sub-product of the study "Preterm SAMBA" which was jointly financed by the Brazilian CNPq (CNPq, Grant 401636/2013-5) and the Bill and Melinda Gates Foundation (Grant OPP1107597).

Competing interests

The author(s) declare that they have not competing interests.

Word count: 2031 words

277

278 **Figure Legend**

279 **Figure 1.** Flow chart of studies identified to be included in the systematic review.

280

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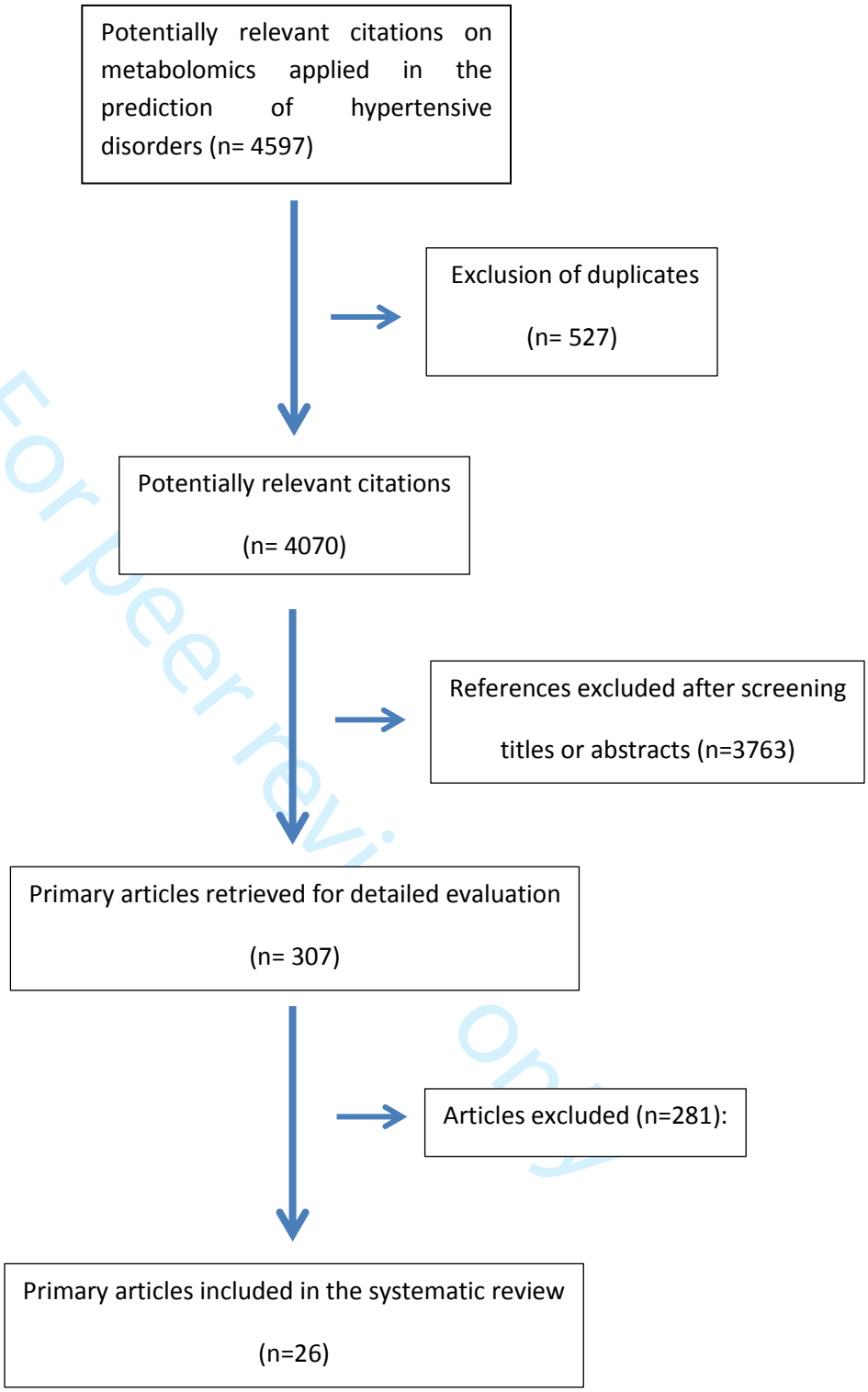

PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - **Moher D, Stewart L & Shekelle P:** Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 **5**:15

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	x☐	☐	3
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	x☐	
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	x☐	☐	52
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	x☐	☐	5,6,7,8
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	x☐	☐	347-353
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	☐	x☐	
Support					
Sources	5a	Indicate sources of financial or other support for the review	x☐	☐	344-345
Sponsor	5b	Provide name for the review funder and/or sponsor	x☐	☐	344-345
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☐	x☐	
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	x☐	☐	54-91

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	x☐	☐	94-99
METHODS					
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	x☐	☐	107-109; 128-132; 140-143
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	x☐	☐	104-107
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	x☐	☐	109-114
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	x☐	☐	121-122
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	x☐	☐	122-126
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	x☐	☐	184-191
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☐	x☐	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	x☐	☐	145-180
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	x☐	☐	193-204
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☐	x☐	
	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 (e.g., I^2, Kendall's tau)	☐	x☐	
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☐	x☐	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	x☐	☐	206-219

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☐	x☐	
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☐	x☐	