MATERNAL INFECTIONS AND DEVELOPMENT OF PREECLAMPSIA: A SYSTEMATIC REVIEW OF THE EPIDEMIOLOGICAL LITERATURE

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ABSTRACT

MATERNAL INFECTIONS AND DEVELOPMENT OF PREECLAMPSIA: A SYSTEMATIC REVIEW OF THE EPIDEMIOLOGICAL LITERATURE

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We systematically reviewed the associations between *H. pylori* (HP), cytomegalovirus (CMV) and *C. pneumoniae* (CP) infection in pregnancy and preeclampsia (PE), a disorder found in 5-8% of pregnancies and a leading cause of maternal and perinatal mortality.

We used Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines and searched PubMed, EMBASE and Web of Science. We also assessed the studies for risk of bias by utilizing a modified version of A Cochrane Risk of Bias Assessment Tool for Non-Randomized Studies of Interventions (ACROBAT-NRSI).

After exclusions based on criteria from these tools, 16 studies were reviewed in detail, of 1,031 initially identified by our search algorithm. Evidence of infection was based on serology (12 studies), Polymerase Chain Reaction (2) or both (2). All four studies of the association between HP and PE found significant odds ratios ranging from 2.7 – 9.2. Two of four studies of CMV and PE found significantly elevated odds ratios (1.9 and 2.7), while only three of ten studies of CP found significant odds ratios, ranging from 3.1 to 4.1. Not all studies controlled fully for confounding, and ten studies were at serious risk of bias.

The available literature provides partial support for the association between these infectious agents and PE, especially for HP, but more rigorous studies are needed in this area, because more than half of the studies examined were at high risk of bias.

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KEY TO ABBREVIATIONS

ACOG	The American College of Obstetrics & Gynecology
ACROBAT-NRSI of Interventions	A Cochrane Risk of Bias Assessment Tool for Non-Randomized Studies
ACS	Acute Coronary Syndrome
adjOR	Adjusted Odds Ratio
BMI	Basal Metabolic Rate
BP	Blood Pressure
CagA	Cytotoxin-associated protein
CC	Case control
CDC	The Center for Disease Control and Prevention
CMV	Cytomegalovirus
CP	*Chlamydophila pneumoniae (C. pneumoniae*)
DNA	Deoxyribonucleic Acid
ELISA	The enzyme-linked immunosorbent assay
EMBASE	Excerpta Medica dataBASE
EVT	Extra-villous Tissue
HLA	Human Leukocyte Antigen
HP	*Helicobacter pylori (H. pylori)*
Hsp	Heat Shock Proteins
IgA/IgG/IGM	Immunoglobulin A/ Immunoglobulin G/Immunoglobulin M
IL	Interleukins
INF	Interferon
ISSHP	The International Society for the Study of Hypertension in Pregnancy
MCH	Major Histocompatibility Complex

MeSH	Medical Subject Headings
MIF	Micro-immunofluorescent
mm Hg	Millimeter of Mercury
mmol	Milli mole
NHBPEP	The National High Blood Pressure Education Program
NK	Natural Killer
NS	Non-significant
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PE	Preeclampsia
PlGF	Placental Growth Factor
PRISMA	The Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RoB	Risk of Bias
RR	Risk Ratio
sEng	soluble Endoglin
sFlt-1	soluble fms-like tyrosine kinase 1
STROBE	Strengthening the reporting of observational studies in epidemiology
Th1/Th2	T Helper cell 1/ T Helper cell 2
TNF	Tissue Necrotic Factor
UreC	Urease subunit C of *H. pylori*
UreE	Urease subunit E of *H. pylori*
VacA	Vacuolating cytotoxin protein
VEGF	Vascular Endothelial Growth Factor
VT	Villous Tissue
WGO	The World Gastroenterology Organization

WHO	THE World Health Organization
WoS	Web of Science

INTRODUCTION/BACKGROUND

Preeclampsia (PE) is one of the leading causes of perinatal morbidity and mortality in the world (1). About 10-15 % of direct maternal deaths in both developing and developed countries are attributed to PE or its complications (2-5). This suggests that once the chain of events related to PE starts it becomes difficult to stop and to reverse the pathologic cycle, especially in the more severe categories of disease.

Maternal deaths are not the only fatal sequel of PE; a quarter of stillbirths worldwide have also been found to be related to PE (6). In addition to effects on mortality, there is substantial morbidity from PE. Some women may develop seizures, which signal progression to eclampsia. Other acute consequences can include stroke, renal and hepatic failure and coagulopathy. These conditions may require intensive care. Apart from these immediate effects, even when the woman apparently recovers uneventfully, there may be both short and long term health consequences. Women may be at an elevated risk for hypertension and cardiovascular disease in later life (7), and newborns may suffer growth retardation and have higher risks of cerebral palsy and other neurodevelopment disorders later (8).

Preeclampsia is most commonly diagnosed when elevated blood pressure (140/90 mmHg or higher, measured on more than one occasion) occurs after 20 weeks of gestation and is accompanied by proteinuria (300 mg or more of protein in a 24-hour urine sample). In unusual cases, the diagnosis can be made in the absence of proteinuria, when hypertension is accompanied by one or more of the following conditions: thrombocytopenia, renal or hepatic failure, pulmonary edema and cerebral or visual symptoms (9).

Research on the origins of preeclampsia, primarily from developed countries, has explored genetic, dietary, metabolic, environmental and cardiovascular risk factors (10-12). However, no cause has been identified yet. The World Health Organization (WHO) Global Survey on Maternal and Perinatal Health has identified some risk factors which include: older age (more than 30 years), low literacy, high body mass index, nulliparity, chronic hypertension, diabetes, cardiac and renal diseases (13).

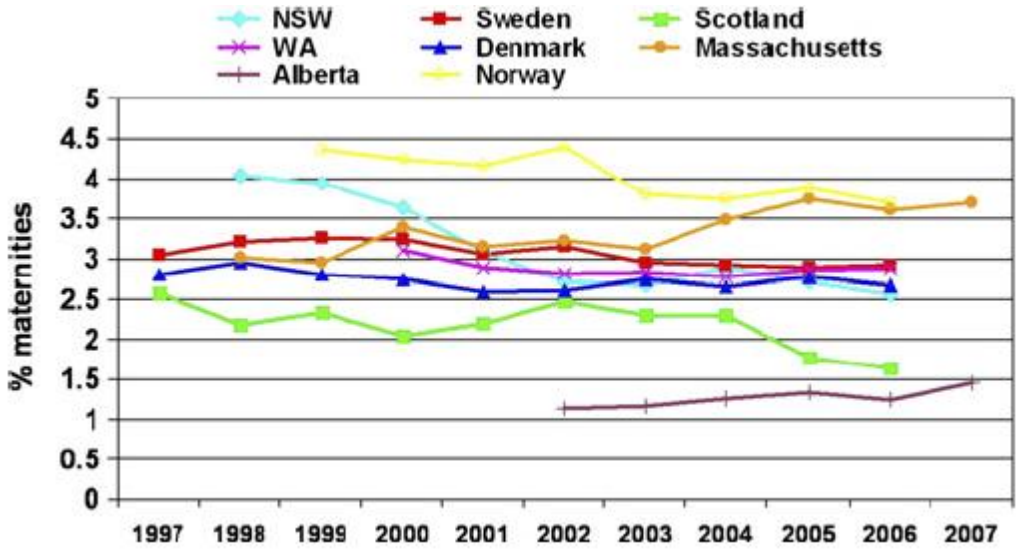
Over the past few decades, efforts have been made to explore the possible role of infections in the development of preeclampsia. However, the scientific and health communities have yet to produce a strong evidence to establish a clear link between infections and the development of preeclampsia.

Descriptive epidemiology of preeclampsia

The burden of preeclampsia varies across different regions. The World health Organization (WHO) estimates the incidence of PE around the world as between 2-10 % of all pregnancies (14). The frequency is almost always low in high income countries as compared to low income countries. Again, the prevalence fluctuates from 1.4-4.0 % across high income countries as shown in the (15).

The low income countries exhibit a wider range. In Africa, PE incidence ranges from 1.8-7.8 % of pregnancies excluding Nigeria where preeclampsia presents with the highest frequency, 1.8-16.7 % (16). A WHO report found that PE incidence was 7 times higher in low income compared to high income countries (14).

Figure 1: Trends in preeclampsia incidence across high income countries.



Source: Roberts et al, 2011 (15).

Evidence is lacking on the trends of preeclampsia especially in low income countries. Information is also not available for all high-income countries. Figure 1 provides some information on time trends for PE incidence for a selective group of countries. This figure shows that over the previous two decades, the frequency of preeclampsia remained almost constant in Sweden, Denmark and Western Australia but it fluctuated for others. Since 2003, the rates have been different in different countries. Rates went down in Scotland, minimally increased in Alberta, Canada, while rates significantly increased in Massachusetts, USA from 2.5 % in 1987 to 3.2 % in 2004 (17).

Infection during pregnancy

Evidence of infection during pregnancy varies across the countries as well as for different organisms. Moreover, status of infection is also not similar for all organisms; there may be primary episode or chronic infection with the development of immune status. According to the CDC, 1-4 % of the pregnant women get CMV primary infection in the US (18). However,

higher estimates are found for primary CMV infection in other countries, especially low-income countries such as Nigeria where the overall seroprevalence was very high with prevalence levels (based on IgM) reaching to 92% or higher (19,20). *H. pylori* has also been found to be present in pregnant women with high prevalence; the burden ranges from 20-80 % across the globe (21).

Risk Factors for the Development of an Atherosclerotic Plaque

Major Factors

- Unhealthy blood cholesterol, hypertension, smoking, insulin resistance, diabetes, obesity and low physical activity

Minor Factors

- Sleep apnea, stress and alcohol.

Clinical Manifestation of Atherosclerosis

Atherosclerosis can affect any vessel in the body; however, the three main categories are significant. These are coronary heart disease, cerebrovascular disease and peripheral vascular disease. The coronary heart disease is the result of atherosclerosis in the coronary arteries, while carotid arteries atherosclerosis results in cerebral or neural manifestation. The peripheral vascular disease may result in the involvement of a variety of organs and systems such as liver, kidney and limbs (22).

Pathophysiology of Preeclampsia

Preeclampsia (PE) is a disorder involving multiple organ systems, and its possible causative factors have been explored from various perspectives, including genetic, dietary and metabolic, environmental, infectious and cardiovascular factors (10-12). But the central factor for the development of PE is the placenta, and after placental delivery, the condition of the woman with PE improves (23). The initiating event in PE is thought to be placental hypoperfusion resulting in placental ischemia. Among several different explanations for the development of PE, three mechanisms have been cited most frequently. These are: lack of remodeling of spiral arteries; maternal endothelial response and coagulopathy and placental factors.

Mechanisms for Development of Preeclampsia

Lack of remodeling of spiral arteries

In normal pregnancies, cytotrophoblasts are responsible for widening of the lumen of spiral arteries by a process which replaces the endothelial layer after they invade these vessels. This allows a marked increase of blood flow to the placenta and to the growing fetus. In PE, however, cytotrophoblasts do not invade spiral arteries deeply enough to convert these arteries to the dilated vessels which are necessary for the maintenance of uninterrupted blood supply to the fetus. This results in placental and fetal hypoxia and under-nutrition. However, whether hypoxia is the result or the cause of the superficial invasion of spiral arteries is still not entirely clear. But in any case, the narrowing of the vessels supplying the placenta cannot maintain the needed blood supply to the fetus, setting the stage for the next steps leading to the development of PE. These processes can begin before the end of the first trimester, and are therefore especially invoked as the mechanism behind early onset PE (24).

Maternal endothelial response and coagulopathy

Possibly in response to the changes described above, PE is characterized by an important maternal endothelial response. As far as the clinical features of PE are concerned, it is the response of maternal endothelium which corresponds with these features. The endothelial reaction results in increased vascular permeability which explains the proteinuria and edema. The change in vascular tone can underly the development of hypertension. Another feature of PE is the production of pro-coagulants which promote hyper-coagulopathy (25).

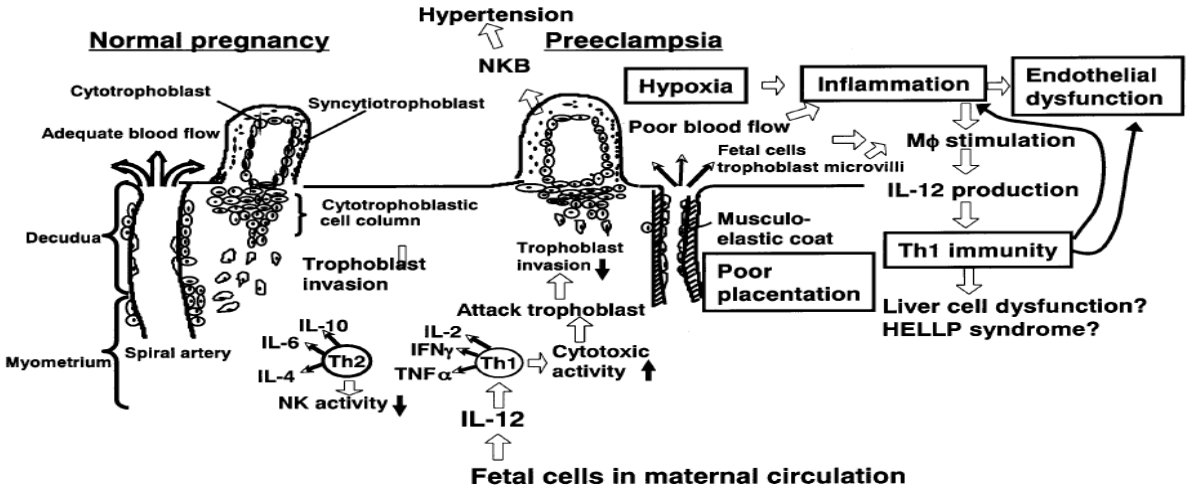
Several markers of endothelial injury are found during this phase. These markers are normally found in pregnant women (26). However, when PE develops, an abnormality in the concentrations of these factors appears. The factors which are favorable for normal pregnancy, like prostacyclin, are reduced in concentrations while the factors which promote vasoconstriction or hyper-coagulation, such as endothelin-1, von Willebrand factor, circulating cellular fibronectin, and thromboxane are increased. Moreover, the responsiveness of preeclamptic women to vasopressors is increased as compared to women with normal pregnancy (27). Also, these features show impairment in the endothelial-derived relaxation of vessels (28, 29).
The maternal endothelial response induces hypertension due to high levels of vasoconstrictors and low levels of vasodilators. The probability of coagulopathy is increased as a result of enhanced concentrations of clotting factors. In more severe situations, a higher consumption of platelets in the process of coagulation may result in thrombocytopenia (30).

Th1 and Th2 imbalance

Two variants of the T cell, Th1 and Th2, are involved as immune mediators in pregnancy. In normal pregnancy, Th2 predominates, and is responsible for the production of different types of interleukins (ILs) such as, IL-4, IL-5, IL-6, IL-10, IL-13 and antibodies. Th2 and the interleukins produced promote normal pregnancy. In contrast, Th1 cells, which produce IL-2,

tissue necrotic factor (TNF)-β, interferon (INF)-γ and induce cellular immunity, dominate the situation in women with PE (31).

Figure 2: Pathological changes in preeclampsia with inflammatory cells.



Up-regulation of Th1 results in cytotoxic activity due to stimulation of natural killer (NK) cells and CD-8 positive T cells (31). Experimental evidence suggests that cultures of peripheral blood mononuclear cells in the blood of preeclamptic women resulted in high concentrations of TNF-β, IL-2 and INF-γ. But the blood of normal women did not show any increase in these factors. This experiment supports the association between PE and these factors (32). Additionally, Th1 has been found to be associated with an increase in endothelin-1 and reduction in plasminogen activator inhibitor-2 (33, 34). These factors are both important mediators of different phases of PE, especially hypertension.

Placental factors

Apart from issues related to the development of spiral arteries and endothelial injury, some other biomarkers have also been extensively studied and are hypothesized to play important roles in the causation of PE. These biomarkers are derived from the placenta. These factors too are normally present in pregnancy. However, there is a reversal in the concentration of these factors.

Factors which are in high concentration in normal pregnancy, such as placental-like growth factor (PlGF) and vascular endothelial growth factor (VEGF), are reduced in preeclamptic women. These factors keep blood flow and placental condition within normal limits (23, 27).

On the other hand, factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble Endoglin (sEng) are also placental in origin but their concentration corresponds to the existence and severity of the PE. These are potent antagonists of PlGF and VEGF. These factors bind to PlGF and VEGF and thus reduce the concentration of free PlGF and VEGF circulating in the blood. This induces endothelial dysfunction. Endothelial dysfunction results in reductions in prostacyclin and nitric oxide concentrations leading to the vasoconstriction and eventually hypertension. At the same time, there is increased production of endothelin, cellular fibronectin, thrombomodulin and von Willebrand factors (27).

The imbalance between two types of markers - angiogenic (PlGF, VEGF) and antiangiogenic (sFlt-1) - as a result of endothelial dysfunction seems to be part of the development of PE; sFlt-1 production takes place about 5-6 weeks before the onset of PE (23).

Infection and atherosclerosis

Some of the biomarkers discussed in the previous section such as, IL-6 and IL-10 are considered indicators of inflammatory responses including infections (35). Based on such evidence, the role of different infections agents including *Chlamydophila pneumoniae (C. pneumoniae), Helicobacter pylori (H. pylori)* and cytomegalovirus (CMV), in the development of atherosclerosis has been explored by the research community (36-44).

Several factors contributed to the selection of these three microorganisms for conducting this review:

1) High frequency and chronic infection or re-infection in the population:

A review conducted by Grayston concluded that the presence of *C. pneumoniae* IgG antibodies remains low (less than 10%) up to the age of 10 years followed by an abrupt increase to attain a level of 50% and then it increases steadily over the rest of life reaching up to 80% (45).

According to the World Gastroenterology Organization (WGO) prevalence of *H. pylori* is higher in developing countries where it reaches up to 85-90 % while across the globe it is 50% (46). According to the Center for Disease Control and Prevention (CDC) Atlanta, more than 50% of the US population is infected by CMV by the time they reach to 40 years of age; this prevalence may go up to 80% (47).

2) Potential role of these micro-organisms in the development of atherosclerosis (as discussed below);
3) Different studies conducted to assess if any association exist between these micro-organisms independently or in combination with the development of PE. The findings of these studies were mixed and in-conclusive; some showed a significant association (21, 48, 49) others no association (50, 51).

A large body of literature is available on the role of infections caused by these micro-organisms and atherosclerosis. In one study of 40 subjects explored the coronary artery specimen and found 22 specimens with acute coronary syndrome (ACS) where *C. pneumoniae* was found in significantly higher concentrations per millimeter of the specimen as compared to 18 non-ACS specimens (36). Another study investigated the presence of *C. pneumoniae* in the atherosclerotic plaques of 76 patients who presented with unstable angina and 75% of the plaques revealed presence of *C. pneumoniae* DNA (37). A nested-case control study compared the presence of antibodies to *C. pneumoniae* in the serum sample with the presence of *C.*

pneumoniae DNA in the coronary artery atherosclerotic plaque (38). Higher proportions of plaques showed the evidence of *C. pneumoniae.*

With regards to *H. pylori*, one study looked for an association between inflammatory markers such as IL-8 which are involved in the process of atherosclerosis development and *H. pylori* (39). The findings showed a significant association between IL-8 and *H. pylori* infection. The subjects with carotid intima-media thickness (IMT) had significantly higher levels of IL-8 than those without IMT. A follow up study with *H. pylori* infected and non-infected individuals found a significant association between infection and carotid artery plaque formation as compared to the non-infected subjects (40). In addition, a meta-analysis of 13 studies reported a significantly higher risk of ischemic stroke in *H. pylori* infected subjects as compared to non-infected ones (41).

The role of CMV in the development of atherosclerosis has been supported by the animal model as well as in humans. Research into an animal model found that a higher proportion of CMV-infected mice showed an evidence of atherosclerotic plaque presence as compared to the control group (42). Another study conducted in Iran reported that the subjects with a higher prevalence of coronary artery syndrome had a significant association with CMV and the virus was found in the atherosclerotic plaque (43). Similarly, a study conducted in India found significant association between CMV-infected young patients and the development of coronary artery disease (44).

Based on the findings from available literature on the role of infections in the development of atherosclerosis, we propose an analogy between the atherosclerotic process of preeclampsia and the atherosclerosis in coronary and cerebral blood vessels because both pathologic processes include evidence of an inflammatory response to endothelial injury. After

injury to the endothelium of vessels in the placenta, such as occurs in preeclampsia, a series of events ensue, including fibrinoid necrosis of the vessel walls, movement of mononuclear cells to the surrounding tissue of the vessel, and accumulation of lipid laden macrophages and presence of lipoproteins in more than normal concentration (52).

Using the association of infection and atherosclerosis as a basic model, a relationship between infection in pregnancy or earlier in life and the subsequent development of preeclampsia has already been explored by the research community. A literature search retrieved two systematic reviews looking at the role of maternal infections and PE (53, 54). These reviews provided opposing results and since then more research work has been published, so we planned to carry out a systematic review focusing on these three infectious agents and PE. Conde et al conducted the review in 2007 to explore the association between maternal infections and preeclampsia (53). Their review included 49 studies and found a significant association between urinary tract infections as well as periodontal disease and the development of preeclampsia. They did not find association between *C. pneumoniae*, CMV, *H. pylori*, and treated or untreated HIV.

In contrast, Rustveld et al reviewed 16 studies to investigate relationship between maternal infections and PE. Their findings showed that some studies were significantly associated while others were not as far as viral and bacterial infections and PE is concerned, including *C. pneumoniae* (54).

OBJECTIVES

1. To identify all observational studies on the association between *Chlamydophila pneumoniae, Helicobacter pylori* and cytomegalovirus (CMV) and preeclampsia; and
2. To carry out a systematic review to determine whether there is an association between these microorganisms[1] and development of PE.

[1] Microorganisms are represented by either detection of their DNA or antibodies against their proteins/antigens.

METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines to carry out this systematic review (55). This review explored the scientific literature to investigate the role of three infectious agents, *H. pylori*, CMV and *C. pneumoniae*, in the causation of preeclampsia in singleton pregnancies. The studies were identified by searching the electronic databases (discussed below), contacting the authors and manually searching the reference lists of the selected studies.

For this purpose, we searched PubMed, EMBASE and Web of Science (WoS), limited to papers in the English language and involving human subjects without any restrictions related to regions of the world, where a study was conducted, or time period.

The main terms searched are enlisted as Appendix B. These terms were combined with a broad set of organisms and related infectious diseases including: all bacteria, all viruses, bacterial infections, or virus diseases. The Appendix C provides search terminology used for Pubmed search engines.

The agents of specific interest - *Helicobacter pylori*, *Chlamydophila pneumoniae* and cytomegalovirus – were included in the broader organism searches of bacteria and viruses, and were also searched as title words to ensure that nothing of interest was missed.

We included all the studies which used cross-sectional, case control or cohort study designs that examined the association between three microorganisms described above and preeclampsia. Eligible studies included those with 1) original data; 2) which used an appropriate and acceptable definition of preeclampsia in a way that it was in compliance with the recognized definition of preeclampsia that has been recommended by relevant organizations or societies such as the American College of Obstetrics and Gynecology (ACOG), the International Society

for the Study of Hypertension in Pregnancy (ISSHP), and the National High Blood Pressure Education Program (NHBPEP) and 3) that described the techniques of detection of infectious agents or antibodies, such as using PCR technique for the identification of DNA or serological testing to determine the presence of antibodies and 4) and finally, those used at least one of the three microorganisms as an exposure and described the methodology of their detection.

The following literature was excluded: case reports, case series, studies with only abstract, commentaries, letters to editors, studies in language other than English. There was no restriction of time period applied on for searching the studies.

Data Abstraction Process

We developed a data abstraction form to collect the required information from the selected studies. This form was refined after the pilot-testing of 5 randomly selected studies not the part of the final study sample. The information obtained through these forms focused on the criteria for study eligibility, methods used, exposure and outcome assessment, confounders and results. Apart from obtaining the information on the variables mentioned above, we also extracted the information on association between the microorganism (s) and PE. For this purpose, we extracted both the crude and adjusted Odds Ratio (OR); when we found only crude OR we took only that value. We calculated OR for the studies which did not calculate it.

Methodology for Quality Assessment

We could not use the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (56). This statement provides guidelines for reporting observational studies and at the same time makes emphasis that this resource is not appropriate to be used for the quality assessment of observational studies. Alternatively, we assessed the risk of bias (RoB) for the studies included in the review as a proxy measure for the assessment of quality of the studies. For the assessment of RoB, we modify A Cochrane Risk of Bias Assessment Tool: for Non-Randomized Studies of Information

(ACROBAT-NRSI) (57). This tool is for studies on interventions but our corresponding variable was the exposure (of infectious agents) so we replaced 'interventions' with 'exposure' in our analysis and text.

The guidelines in ACROBAT-NRSI suggest that the tool should be followed right from the design stage. Unfortunately, this tool was published after we had already finished our analysis. However, we still had the opportunity to assess the RoB by re-analyzing the related items. In the next stage, as recommended by this tool, the review of each individual study is provided. This review was conducted utilizing the following seven domains to identify any bias which may exist:

1. Bias due to confounding
2. Bias in the selection of participants into the study
3. Bias in measurement of interventions/exposure
4. Bias due to departures from the intended interventions/exposure
5. Bias due to missing data
6. Bias in measurement of outcome
7. Bias in selection of the reported results.

Each domain has certain questions with options of responses as yes, probably yes, no, probably no and no information. Based on the responses to the questions each domain may receive one of the four levels of bias: low risk, moderate risk, serous risk and critical risk of bias.

For each study, the overall risk of bias was assessed by the cumulative assessment for all the seven domains. The final RoB for each study is also assessed at the same scale as for each domain. The tables/checklist to show these domains with analysis for each study is attached as Appendix D.

RESULTS

Descriptive Analysis of Review

The initial attempt with Pubmed, Embase and Web of Science search terms retrieved 1,031 articles. Further analysis of the titles and abstracts of the articles excluded 1,014 search results based on exclusion criteria as shown in figure 1. After reviewing the reference lists of the remaining 14 articles, two more were added to achieve a final number of 16 articles which resulted in obtaining information about 5,614 women. These studies looked for the association between at least one of the three microorganisms, *C. pneumoniae, H. pylori* and cytomegalovirus (CMV), and development of preeclampsia (PE). The search and selection process of studies is provided in a figure as Appendix E.

Sources of the Studies

Eight countries contributed to the data in this review. Three studies each were conducted in Canada, Turkey and the USA while two studies each were from Italy and the UK. One study each was conducted in Finland, Norway and Venezuela. All but one study was carried out in North America and Europe; one was done in South America. All but two studies (one nested case - control and the other a cohort study) used the case control design.

Fifteen studies used the case-control (CC) design and one was carried out as cohort study. Including the cohort studies, three were population based while the rest of them (13) were conducted in hospital setting. The controls were pregnant women who did not have the features of preeclampsia. The hospital studies included the women who were visiting hospitals for antennal care or were admitted either to get services for an emergency condition or to deliver in the hospital.

Nine of the sixteen studies provided information on controlling for confounding variables. Three studies controlled for confounding variables at design phase by using a matched design while six controlled confounding variables at analysis phase by constructing a logistic regression model. Most of the studies controlled for parity and maternal age while using matching or adjusted analysis.

Eight articles exclusively looked at the role of *C. pneumoniae.* Six of these studies assessed the evidence of *C. pneumoniae* by looking at antibodies, one looked for *C. pneumoniae* using PCR, and one study used both techniques.

Three articles each explored an association between PE and *H. pylori* and CMV. The researchers of these studies utilized different methods to assess the presence of these organisms, such as: antibodies against the microorganism or carrying out PCR or extracting the DNA. One study each assessed the presence of CMV or *H. pylori* through PCR/DNA and the remaining two studies each assessed evidence by using antibodies. Two articles studied two microorganisms as exposures for the development of PE; one looked at *C. pneumoniae* and CMV, while the other explored the role of *C. pneumoniae* and *H. pylori.*

Preeclampsia Definitions and Diagnosis

All studies except one (58) provided information on how the blood pressure (BP) of pregnant women was assessed in making the diagnosis of preeclampsia. Thirteen studies checked BP twice for making a diagnosis of hypertension. Six studies measured BP twice six hours apart, and four measured BP twice four hours apart. The remaining three studies did not provide information on the interval between BP assessments taken at two points. Four studies divided preeclampsia into mild and severe categories based on both BP readings and protein levels in the

urine; two studies made sub-groups of early and late onset of preeclampsia based on the timing of development of the clinical features.

Table 1: Recommendation of ACOG for the diagnosis of Preeclampsia.

Blood Pressure	• Greater than or equal to 140 mm Hg systolic or greater than or equal to 90 mm Hg diastolic on two occasions at least 4 hours apart after 20 weeks gestation in a woman with previous normal blood pressure. • Greater than or equal to 160 mm Hg systolic or greater than or equal to 110 mm Hg diastolic, hypertension can be conformed within a short interval (minutes) to facilitate timely hypertensive therapy.
And	
Protein	Greater than 300 mg/24 hours urine collected (or this amount extrapolated from a timed collection). Or Protein/creatinine ratio greater than 0.3 (both measured in mg/dl). Dipstick recording of 1+ (useful only if other quantitative methods are not available).
Or in the absence of proteinuria, new - onset hypertension with the new onset of any of the following	
Thrombocytopenia	Platelet count less than 100,000/ml
Renal Insufficiency	Serum creatinine concentration more than 1.1 mg/dl or a doubling of the serum creatinine concentration in the absence of other renal disease.
Impaired Liver Function	Elevated blood concentration of liver transaminases to twice normal concentration.
Pulmonary Edema	
Cerebral/Visual symptoms	

All but one study used a cut off value of 140/90 mm Hg for diagnosing mild hypertension while 160/110 mm Hg was taken as the cut off value for severe hypertension which correspond to mild preeclampsia and severe preeclampsia respectively. Four studies explicitly mentioned following the American College of Obstetrics and Gynecology (ACOG) guidelines as summarized in table 1(59), while one study each mentioned following guidelines of the International Society for the Study of Hypertension in Pregnancy (ISSHP), narrated in table 2 (60) and National High Blood Pressure Education Program (NHBPEP). One study took only diastolic BP as the criterion for diagnosing preeclampsia (61). One study did not provide any criterion for diagnosing PE, but stated that they enrolled preeclamptic women as cases (58).

Proteinuria was assessed by two methods, either measuring proteins in 24 hours-collected urine or using urinary dipstick findings. All studies that obtained 24-hour urine samples used the same level of proteins in urine (>300 mg/24 hours) for mild preeclampsia. Studies that subdivided their sample into mild and severe PE also agreed upon one value of >500mg /24 hours. However, for dipstick methods, studies differed. For mild PE, dipstick 1+ and 2+ were used; in comparison either dipstick 3+ or 4+ was used for severe PE by different studies.

Table 2: Recommendations of ISSHP for the diagnosis of Preeclampsia.

Blood Pressure	• De Novo hypertension after gestational week 20
And the new onset of one or more of the following	
Protein	≥ 300 mg/day or a spot urine Protein/creatinine ratio ≥ 30 mg/mmol
Renal Insufficiency	Serum creatinine ≥ 0.9 mmol/L or Oliguria
Neurological Problems	Convulsions (eclampsia), hyper-reflexia with clonus, severe headache with hyper-reflexia, persistent visual disturbances (scotomoa).
Hematological Disturbances	Thrombocytopenia, Disseminated Intravascular Coagulation (DIC) and Hemolysis.
Fetal Growth Restriction	

The timing of physical examination and biological information collection was similar in almost all studies. Except for four studies, the rest did not provide information on the semester during which the biosamples were collected.

The diagnosis of preeclampsia was based on the findings of hypertension and proteinuria assessed after the 20th weeks of gestation. Six studies explicitly mentioned that they followed some guidelines in the diagnosis of PE; four followed ACOG, one each followed ISSHP and NHBPEP.

The risk of bias (RoB) analysis found that more than six studies were at the lowest level of risk, low risk (21, 49, 50, 61-63) and one study was at moderate risk of bias (64). More than

half of the studies reviewed were either at serious risk (48, 65-68) or critical risk of bias (51, 58, 69, 70).

Association between Micro-organisms and Preeclampsia

To determine the evidence of an infectious agent, usually two types of tests are used: serological tests which show the antigen-antibody reaction and extraction of DNA of an infectious agent in body tissues. All studies we reviewed used serological tests but four also used tests for the detection of microorganism DNA from the placental tissue.

All studies explored the association between PE and these micro-organisms either for all or at least for one. Five studies did not calculate the OR so we calculated it based on the provided data. Maternal age was the most commonly used matching variable.

Serological Tests

These tests are based on antigen-antibody reactions. The serological tests have been named according to the type of antigen-antibody reaction/mechanism, of complex formation (71). Different types of testing strategies come under this main domain. However, below is a brief description of the two tests utilized in the studies which are investigated in this review.

The Enzyme-Linked Immunosorbent Assay (ELISA) is one of the techniques which follow the principle of antigen-antobody reactions. In this test, the antibodies change color when a substance reacts with them. Antigens from the serum of patients are attached to a surface to which a specific antibody is added which binds to the antigen. The added antibody is linked to an enzyme; the substance which contains the substrate for that enzyme is added as a final step. The reaction usually changes the color of the substrate.

Another test which utilizes the serological techniques is the Micro-immuno Fluorescence (MIF) Test. It is a specific type of serological tests utilized for some micro-organisms including *C. pneumoniae.* This test uses an indirect fluorescent antibody (FA) technique which helps in observing the binding of antigen antibody. This is facilitated by anti-globulin which is

fluorescein-conjugated and corresponds to specific antibody molecules. The antigen used in this test comes from whole elementary body C*hlapmydophila* organisms. These elementary bodies are grown in cell culture, purified and then treated with 0.05% formalin. This preparation of antigens can be stored in refrigerator for many years (72).

Tests for the detection of DNA

For the detection of DNA from placental tissue, a Polymerase Chain Reaction (PCR) test is applied. The PCR test is a laboratory technique through which not only the DNA is detected but it is amplified by making millions of copies from a single strand or a few copies of DNA (73). To carry out a PCR test, a nucleic acid target source (micro-organism) is required along with small DNA primers for the amplification of the 5` and 3` ends, the enzyme polymerase and an appropriate instrument to regulate the temperature during different phases of the test.

One study utilized the PCR test to detect CMV DNA by using the genetic information present in the Major Histocompatibility Complex (MCH). The MCH is a collection of molecules on the cell surface which is coded by a large gene family; this controls the major part of the immune system (74). Its gene family has been divided into three subclasses, class I, class II and class III. The class I and class II are also called Human Leukocyte Antigens (HLA). There are nine HLA genes which have been studied the most. The studies explored in this review investigated for DRB1, DQA1 and DQB1 and DR7. CMV DNA was amplified by using PCR.

Apart from indicating the evidence of an infection, the level of anti-body titer also provides information on the status of infection. Immunoglobulin A (IgA) is more frequently found in the elderly and the individuals who have chronic infections and IgM is the first antibody to appear in the serum in response to infection due to bacteria, viruses or toxins. Usually, IgG appears in the body when IgM levels start decreasing and then it persists for a longer duration. It also appears as a response to chronic infection (75).

Helicobacter pylori (H. pylori) and Preeclampsia

Most studies focused on using serological methods to identify various types of antigenic structures found in *H. pylori* (Appendix F). The literature discusses a long list of these antigenic structures, however, the most commonly used for diagnostic purpose are: Cytotoxin-associated protein (CagA), the vacuolating cytotoxin protein (VacA), urease subunits, flagellin subunits and heat-shock proteins (HspA and HspB) (21).

As shown in tables 3 and 4, four studies (21, 51, 64, 65) looked at the role of *H. pylori* in the development of preeclampsia. One study explored antibodies produced in response to different antigens of *H. pylori* by using serological methods as well as evidence of *H. pylori* DNA using the PCR test (64) while the remaining three studies explored only antibodies (21, 51, 65). Of these three latter studies, one looked at antibodies produced in response to both *H. pylori* and *C. pneumoniae* (51) while the other two for *H. pylori* only (21, 64). All the studies which looked at the association between antibodies and PE found a statistically significant association: [Adj OR=9.2 (2.8-30.0)] (21), [OR=3.8 (1.2-11.8)] (53), [OR=2.9 (1.1-7.8)] (65) and [OR (CagA) 26.04 (8.19-82.73)] but placenta test for DNA extraction was found to be negative (64).

Cytomegalovirus (CMV) and Preeclampsia

Four studies examined the relationship between CMV and PE each used a case-control design (48, 50, 63, 66). Two of the studies looked at the association between anti-CMV antibodies, IgA, IgG and IgM and PE (48, 50). Another study looked at the association of PE with antibodies against both *C. pneumoniae* and CMV (63) while the fourth explored the association between the presence of CMV DNA in peripheral blood and PE (66).

The study that investigated an association of PE with C. *pneumoniae* and CMV simultaneously made a distinction between early onset PE (diagnosed during 20-34 weeks

gestation) and late onset PE (diagnosed after 34 weeks gestation) (63). Both the micro-organisms were found as risk factors for the early onset PE [OR_{CMV}=2.1(0.3-17.1); OR_{CP}=1.8(0.4-7.4); OR_{BOTH}=2.9(0.7-12.2)]. In contrast, the two microorganisms were protective for late onset PE [OR_{CMV} = 0.6 (0.2-1.5); OR_{CP} = 0.5(0.2-1.3); OR_{BOTH} = 0.6(0.2-1.4)].

The remaining two studies found a significant association between the evidence of infection with CMV and PE (48, 66). One investigated CMV in peripheral blood assessed by using PCR (66). As compared to the controls, preeclamptic women had a risk of 7.15 of detecting DR7 ($p<.01$); similarly the risk was also higher among preeclamptic mothers than the controls for DQA1*0201 [RR: 4.9 ($p<.02$) and for DR7 and/or DR06 [RR=8.53 ($p<.0003$)]. The other study explored the association by using serological tests [OR for IgG: 1.9 (1.4-2.7)] (48).

***Chlamydophila pneumoniae (C. pneumoniae)* and Preeclampsia**

Ten studies explored the evidence of infection with *C. pneumoniae* and development of PE (49, 51, 58, 61-63, 67-70). Five of them found a significant association between microorganisms and PE (49, 58, 62, 67, 68). Eight of the ten studies attempted to find only the role of *C. pneumoniae* infection in the development of PE. Of the remaining two studies, one examined the association of *C. pneumoniae* along with CMV and PE (63) and the other study investigated *C. pneumoniae* along with *H. pylori* and PE (53). Similarly, nine studies used serological methods to find out this evidence while one study utilized DNA extraction and one study used both methods to establish evidence of this relationship. All studies were conducted using a case-control design except one, which used a cohort design.

The eight studies which used only serological tests varied in their choice of antibodies against *C. pneumoniae*. Six of them looked for all three antibodies-IgA, IgG and IgM (58, 61, 62, 68-70) while one study investigated IgG and IgM (51) and two others explored for IgG only (49, 63). Out of the two studies which looked for IgG only, just one study found a significant

association between IgG and PE [OR: 5.3(1.4-2.0)] this was carried out by using the cohort study design (49). The other study assessed early (diagnosis of PE between 20-34 weeks gestation) and late onset PE (diagnosis of PE after 34 weeks of gestation) but the association was not found to be significant [OR_{EARLY}: 1.8 (0.4-7.4); $OR_{LATE:}$ 0.5 (0.2-1.3)] (63). The study which looked for IgM and IgG by using the serological test did not find a significant association for both the immunoglobulins [$OR_{IgM:}$ 1.1 (0.4-3); $OR_{IgG:}$ 1.3 (0.5-3.1) (51).

For the rest of the six studies which looked for three immunoglobulins, IgA, IgM and IgG by using the serological tests, two studies showed an association for IgG only; one was significant [OR: 3.1 (1.8-7.9)] (68) and the other was not [OR: 1.1(0.8-1.6)] (62). The other two were at a borderline for a significant association (58, 70). One of them reported estimates for IgG only [OR: 1.6 (1.0-2.6)] (70) while the other reported estimates for all three immuno-globulins. However, there was almost no difference among these estimates for all three immuno-globulins [OR:1.2 (1.0-4.5)] (58). One of the remaining four studies did not report the estimates but mentioned the significance status as 'NS', which means there was not a significant association (69), another was found to be non-significant with protective effects [OR: 0.6(0.2-1.6) (61).

The two studies which detected *C. pneumoniae* by identifying DNA both found supportive evidence. One study tested the correlation between gDNA copy numbers and anti-*C. pneumoniae* which was found to be strongly correlated ($p<.0001$) (62), the other study explored *C. pneumoniae* in placental tissue by using PCR. This study explored both the villous tissue (VT) and extra-villous tissue (EVT); a significant association was found for the combination of VT and EVT with PE [OR: 4.10(1.07-15.62)] but not for EVT only (67).

Table 3: Studies retrieved and reviewed systematically, arranged by the organism investigated using serological tests.

SNo	Author, Year, Country, Ref	Study Design	Final Study Size, No.	Cases with PE, No.	Assessment of Exposure	Timing of collection of Biosamples	Assessment of Outcome	OR	95 % CI	Matching and Adjustment Variables	Quality Assessment (weighted score)
Studies of *Helicobacter Pylori* antibodies											
1	Cardaropoli et al, 2011, Italy (21)	Case-control	66	17	Serum serology for specific Antibodies against HP antigens: CagA, VacA, Urease C, Flagellin, Urease H, Urease A, Urease E.	Before delivery	Records/ clinical	*H. pylori*: 9.2 CagA: 17.7 VacA: 4.9 UreC: 2.8 UreE: 4.4	2.8-30.0 5.3-59.5 1.6-14.7 1.0-7.8 1.6-12.3	Adjusted for: maternal age, pre-pregnancy BMI, parity, maternal and family risk factors	49
2	Aksoy et al, 2009, Turkey (65)	Case-control	83	53	ELISA for anti-*H. pylori* IgG	Antenatal period	Records/ clinical	2.9	1.1-7.8	No information on adjusted variables.	55
Studies of Cytomegalovirus antibodies											
3	Strand et al, 2012, Norway (50)	Nested case control	2,461	1,470	Serum samples for anti-CMV IgA, IgG and IgM	Antenatal period	Records / Clinical	IgG: 0.9 IgM: 1.1	0.7-1.1 0.5-2.4	Adjusted for: mat age, parity, smoking in pregnancy	66
4	Xie et al, 2010, Canada (48)	Case-control	187	78	Serum samples for anti-CMV IgA, IgG and IgM	Antenatal period	Records / Clinical	IgG: 1.9	1.4-2.7	No information on adjusted variables.	55
Studies of *Chlamydophila pneumoniae* antibodies											
5	Chrisoulidou et al, 2011, UK (61)	Case-control	60	30	Fasting blood samples for CP antibodies IgA, IgG, IgM.	No information provided	Records/ clinical	0.6*	0.2-1.6	Matched on: age, parity, week of gestation	50
6	Karinen et al, 2008, Finland (49)	Cohort	1556	77	MIF^ for anti-CP IgG	1st trimester; 10.4 weeks	Records/ clinical	IgG: 5.3	1.4-20	Adjusted for: BMI, smoking, family history of PE.	63
7	Aral et al, 2006, Turkey (58)	Case-control	116	69	ELISA: anti-*C. pneumoniae* IgA, IgG and IgM	No information provided	Records/ clinical	IgA:1.9* IgG:2.1* IgM:2.2*	0.8-4.3 1.0-4.5 0.9-5.1	Adjusted for none, crude analysis.	27
8	Goulis et al, 2005, UK (69)	Case-control	Multiparous 20 Primiparous 33	Multiparous 9 Primiparous 6	Blood specimens for Enzyme Immunoassay for anti-*C. pneumoniae* IgA, IgG and IgM.	16-22 weeks; 28-40 weeks	Records/ Clinical (ISSHP*** guidelines)	Multiparous IgA IgG IgM Primiparous IgA IgG IgM	 NS NS NS NS NS NS	No information on adjusted variables.	48

Table 3 (cont'd)

9	Raynor et al, 2004, USA (70)	Case-control	287	81	ELISA: anti-*C. pneumoniae* IgA, IgG and IgM		Records/ Clinical (ACOG** guidelines)	IgG:1.6	0.9-2.6	Adjusted for: age, parity, seropositivity	60
10	Heine et al, 2003, USA (68)	Case-control	74	37	MIF^ for anti-*C. pneumoniae* IgA, IgG, IgM	At delivery	Records/ clinical	IgA* : 1 IgG*: 3.1 IgM*: 1	NS 1.8-7.9 NS	Adjusted for: age, gestational age.	50
Studies using antibodies of more than one microorganisms											
11	Ustun et al, 2010, Turkey (51)	Case-control	80	40	*C. pneumoniae* IgG, IgM *H. pylori* IgA	Antennal period, fasting sample	ACOG guidelines	*C. pneumoniae** IgG: 1.3 IgM: 1.1 *H. pylori** 3.8	0.5-3.1 0.4-3.0 1.2-11.8	No information on adjusted variables.	45
12	Von Dadelszen et al, 2003, Canada (63)	Nested case-control	Early onset=122; Late onset=142	Early onset=9; Late onset 29	Serology for CMV antibodies and *C. pneumoniae* antibodies	Antenatal period	Records/ Clinical. NHBPEP criteria	Early onset* CMV: 2.1 CP: 1.8 Both: 2.9 Late onset* CMV: 0.6 CP: 0.5 Both: 0.6	0.3-17.1 0.4-7.4 0.7-12.2 0.2-1.5 0.2-1.3 0.2-1.4	Matched om: maternal age (5 years) and parity (0,1,>=2)	53

CP=*C. pneumoniae*

Table 4: Studies retrieved and reviewed systematically, arranged by the organism investigated detecting DNA.

SNo.	Author, Year, Country, Ref	Study Design	Final Study Size, No.	Cases with PE, No.	Assessment of Exposure	Timing of collection of Biosamples	Assessment of Outcome	OR	95 % CI	Matching and Adjustment Variables	Quality Assessment (weighted score)
Studies of *Helicobacter Pylori* DNA											
1	Ponzetto, 2006, Italy (64)	Case-control	86 For PCR Placenta:20	43 For PCR Placentae:10	Serum serology for anti-*H. pylori* IgG and for CagA proteins. PCR for placenta DNA.	5 ml of blood before delivery	Records/ Clinical (ACOG** guidelines)	*H. pylori* : 2.67 CagA : 26.04 ***Placenta test for DNA extraction found NEGATIVE.***	1.08-6.57 8.19-82.73	Matched on: parity	50
Studies of Cytomegalovirus DNA											
2	Carreiras et al, 2002, Venezuela (66)	Case-control	56	27	PCR amplification for CMV detection	NA	Records/ clinical	DR7: 7.15 DQA1*0201:4 .9	P=0.01 P=0.02)	No information on adjusted variables.	50
Studies of *Chlamydophila pneumoniae* DNA											
3	Xie et al, 2010, Canada (62)	Case-control	107	50	ELISA: anti-*C. pneumoniae* IgA, IgG and IgM Venous blood for DNA	Antenatal	Records/ clinical	IgG: 1.14	0.84-1.55	Matched on: maternal age, parity, gestational age at sampling.	51
4	Gomez et al, 2009, USA (67)	Case-control	78	48	Placental tissue for detection of *C. pneumoniae* by using PCR.	1st trimester	Records/ Clinical (ACOG** guidelines)	EVT" & VT'=4.10 EVT"=3.23	1.07-15.62 0.65-16.12	No information on adjusted variables.	49

*OR calculated by reviewer/author; **ACOG=American College of Obstetricians and Gynecologists; ISSHP=the International Society for the Study of Hypertension in pregnancy; MIF^=Microimmunofluorescence; EVT" = Extravillous Trophoblast Cells; VT'=Villous Trophoblast Cells. NHBPEP=National High Blood Pressure Education Program.

Assessment of Risk of Bias

Six of the 16 studies were at low risk (21, 49, 50, 61-63) and one study had moderate level risk (64). Out of the remaining nine studies, five had serious risk (48, 65-68) and four (51, 58, 69, 70) were assessed as at the level of critical risk of bias.

By organisms, out of the four studies which explored *H. pylori* one each was at a risk of the level low (21), moderate (64), serious (65) and critical (51). In comparison, out of the four studies which investigated the role of CMV, two each had a risk of the level low (50, 63) and serious (48, 66). Out of the ten studies on *C. pneumoniae*, two showed serious risk (63, 67) and four each had low (49, 61-63) as well as critical (51, 58, 69, 70) risk of bias. The most common problem with the quality of study was bias due to confounding.

DISCUSSION

Of the sixteen research articles reviewed in this study three of the ten showed a positive association between *C. pneumoniae* and PE. Four of them investigated association between *H. pylori* and PE, and all were found significantly associated. Of the four studies which explored the relationship between CMV and PE, two reflected a significant association between CMV and PE. Our investigation added nine more studies to the review in comparison to the previous two meta-analyses (53, 54); still the overall evidence is not sufficient to provide a clear conclusion except *for H. pylori* and CMV to some extent. To our knowledge, of the previously conducted meta-analyses; one showed an association between infection and PE (54) while the other did not (53). But these two reviews focused on infection almost everywhere in the body while our review is exclusively on three micro-organisms which have been found associated in the development of atherosclerosis (21, 48, 49).

With regards to the studies focused on *H. pylori*, all four studies utilized serological tests for the evidence of *H. pylori* infection (21, 51, 64, 6) and found a significant association between the microorganism and PE; one study additionally looked at *H. pylori* DNA by using PCR in placental tissue but could not establish an association (64). This may be due to the smaller sample size for the PCR study (n=20) as compared to the serological analysis (n=86).

All four studies had a strong association but only one of them was at a low risk of bias (21), the risk of bias (ROB) ranged from moderate (64) to critical or serious (51, 65). The main factor for the increased level of ROB was that these studies either did not provide information on the confounders or did not control all of the potential confounders. Usually, a weak association could be a result of uncontrolled confounders; however caution should be taken before applying these results.

Out of the four studies on CMV, two found a significant association (48, 66). One had a moderate association (48) while the other study did not report a level of association (66). The latter study used DNA extraction method to determine the evidence of infection. However these both studies were at a serious ROB. On the other hand, none of the other two studies was significantly associated (50, 63). One of the latter studies assessed the outcome as early and late onset PE (63). For the early onset PE, the sample size for PE cases was small (n=9) and the late onset it was a bit higher (n=29). For the early onset PE, CMV was reflective of a risk factor (RF) [OR: 2.1(0.3-17.1)] while the late onset PE indicated CMV as a preventive factor [OR: 0.6(0.2-1.5)]. This article used *C. pneumonia* along with CMV as an exposure and the trend of its association with PE was similar to CMV (*C. pneumoniae* as an RF for early onset while preventive for late onset PE). It may suggest that infection was of severe in nature for the early onset PE however it could not reach to a significant level; in contrast the late onset PE was of mild in nature and acted as a preventive factor.

As far as *C. pneumoniae* is concerned, out of the ten studies, three found a significant association between *C. pneumoniae* and PE (49, 67, 68). All three showed a strong association. One of them used PCR techniques to look for the evidence of *C. pneumoniae* DNA in the placental tissue at two levels, extra-villous (EVT) and villous tissue (VT). The association was significant when the samples were assessed for both the tissues as compared to only EVT. In the former case (EVT and VT combined), the number of PE cases was 15 as compared to the latter case (EVT only) where nine cases of PE were found. The low number of PE cases in EVT only scenario might have resulted in no association. However, two of these three studies were found to be at a serious risk of bias, the main factor being no information on controlling for the confounders (67, 68).

The rest of the studies showed an association between *C. pneumoniae* and PE where *C. pneumoniae* was found as a RF (OR>1) (51, 58, 61, 62, 69, 70) except for two studies (61, 63) where *C. pneumoniae* acted as a protective factor. Nine of the ten studies utilized serological tests and all of them looked for IgG; in all of them *C. pneumoniae* was found as a RF (49, 51, 58, 62-63, 68-70) except one (61). As IgG usually indicates chronic infection, it may point out here as well that these studies had an infectious episode during early weeks of pregnancy. However, two studies also found an association with IgM along with IgG (51, 58) which may suggest a recent episode during pregnancy in addition to previous episode(s).

Nine out of the 16 total studies found a statistically significant association between infectious agents and PE; only three of them were of moderate to high quality studies as assessed by the existence of risk of bias. The current body of evidence is too small to reach any clear conclusion to determine the role of infections in the development of PE. Moreover, this review did not find any study from low income countries, and the generalizability of the current findings is also very much limited. The findings of this review are not strong enough to determine the definite association of infection caused by *H. pylori*, CMV or *C. pneumoniae* with that of PE. However, this review has uncovered the need for more research with a robust methodology

LIMITATIONS

Our review had several limitations. Our search was limited to studies published only in the English language; this may have reduced our sample size due to missing non-English language studies. In future, researchers can get the required information from the authors of the articles in languages other than English by personally contacting them.

The quality of the studies in this review varied significantly. More than half of the studies were either at critical bias or serious bias and this made the results questionable.

Two of the studies cited in different reviews and found in the reference lists of some articles could not be accessed.

There was not a single study from low income countries. The number of studies was also not sufficiently large; when considered separately, there were not enough studies for as the recommednde number of studies for categorical outcome is 4 for each group (Cochrane handbook). Therefore, we could not pool the results or carry out meta-analysis as the number of studies for a particular infectious agent was low, or these studies used different methods of analysis (serological tests, PCR) and provided different measures of association.

CONCLUSIONS

This review attempted to investigate an association between *C. pneumoniae, H. pylori* and cytomegalovirus (CMV) and PE and found that just above half of the studies (9 out of 16 found a significant association with at least one of these microorganisms; most of the studies come from high and middle income countries. Since the two previous meta-analyses were conducted, nine more studies on this topic have been added still the available literature does not strongly indicate an association between these infectious agents and PE except for *H. pylori.*

Overall the quality of studies, as assessed by the existence of risk of bias, was not considered to be using a robust methodology. These were at the risk of serious or even more severe bias. Most of the studies were found at a high risk of bias due to inability to report or control for the confounders, the findings of this review guide us for the need of more research on this topic with high quality studies.

APPENDICES

Appendix A: Structured Summary

Background: Preeclampsia (PE) affects 5-8 % of pregnancies and is one of the leading causes of perinatal morbidity and mortality in the world. Presently, no causative factor has been identified for PE. Over the last decade efforts have been made to explore the possible role of maternal infections as a risk? factor for the development of PE.

Objectives: We carried out a systematic review to determine if an association exists between PE and infection with *H. pylori* (HP), cytomegalovirus (CMV) or *C. pneumoniae* (CP).

Data Sources: We systematically searched PubMed, EMBASE and Web of Science for articles in the English language. Additionally, studies were added after manual searches by reviewing the reference lists of the selected articles. Search terms included infections, bacterial infections, viral infections, *H. pylori* (HP), cytomegalovirus (CMV) *C. pneumoniae* (CP), preeclampsia, eclampsia, pregnancy induced hypertension and gestational hypertension.

Study quality was assessed by applying a modified version of the Cochrane Risk of Bias Assessment Tool (ACROBAT-NRSI). Studies were grouped corresponding to the infectious agents evaluated and a synthesis of results was carried out accordingly. Findings were reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines.

Study Selection: Articles included cross-sectional, case control and cohort studies designs. Eligible studies included those with 1) original data, 2) that used a recognized definition of preeclampsia that followed guidelines of relevant organizations or societies (i.e. the American College of Obstetrics and Gynecology (ACOG), the International Society for the Study of Hypertension in Pregnancy (ISSHP), and the National High Blood Pressure Education Program

(NHBPEP), and 3) which described the techniques of detection of infectious agents or antibodies, and 4) included at least one of the three microorganisms of interest .

Data Extraction: A data abstraction form was used to extract and document the required information from the selected articles including the year of publication, the country of publication, type of study design with population base used (hospital or population), the sample size and the adjustment for confounding, whether by matching at design phase or at analysis. give some basic variables here?.

Data Synthesis: We identified 1031 hits after searching the three databases; after reviewing the titles and abstracts we found 15 relevant articles that underwent full text review . All 15 studies met our final inclusion criteria. After review of the reference lists we found 3 additional articles, 1 of which met our final inclusion criteria after full text review , Thus 16 studies underwent data abstraction and were included in the final review .

Fifteen studies utilized the case-control design, one was a cohort. Three of them, including the cohort study, were population based studies and the rest were (13 studies) were hospital-based CCS. All studies used either serological tests, Polymerase Chain Reaction (PCR) tests, or both to determine the presence of infection. Four studies described the exact timing of collection of the samples (2 samples were drawn in the 1^{st} trimester, 1 in the 2^{nd} trimester and 1 at the time of delivery); for the rest of the studies, no information was provided for the timing of collection of samples.

The diagnosis for PE was made based on the assessments made after the 20^{th} week of gestation. Four studies explicitly mentioned following ACOG guidelines while one study followed the guidelines of ISSHP and the other NHBPEP. All studies explored the association between PE and at least one of the three microorganisms.

Four studies explored the association between HP and PE and each found a significant association [Adj OR: 9.2 (2.8-30)], [OR: 2.9 (1.1-7.8)], [OR: 3.8: (1.2-11.8)], and [OR: 2.7 (1.1-6.6)]. Of the four studies investigating the relationship between CMV and PE, two showed significant association [OR: 1.9(1.4-2.7)] and [OR: 7.2 (p.02)] while three out of ten studies found a significant association between CP and PE [OR: 5.3 (1.4-20)], [OR: 3.1(1.8-7.9)] and [OR: 4.1(1.1-15.6)].

Nine of the sixteen studies provided information on controlling for confounding variables; three studies used matched case-control design and six controlled confounding variables at analysis. The variables most commonly matched or controlled at analysis were parity and maternal age. The review also looked at the quality of studies by assessing the risk of bias (ROB) utilizing a modified version of A Cochrane Risk of Bias Assessment Tool: for Non-Randomized Studies (ACROBAT-NRSI). The ROB assessment found six studies at low ROB, nine at serious or critical and one was found to be at a moderate ROB; bias due to confounding was the most common serious flaw.

Limitations: This review did not include articles from languages other than the English. The final number of articles included was low and meta-analysis could not be carried out. Additionally, about 2/3 of the studies were found to be at a high risk of bias.

Conclusions: A small body of evidence exists on the relationship between PE and HP, CMV and CP. The available literature does not indicate an association between these infectious agents and PE except for HP; more than half of the studies were at a high risk of bias. Literature using a more rigorous methodology is needed in this subject area.

Appendix B: Main terms searched

1. Preeclampsia
2. Eclampsia
3. Pregnancy induced hypertension
4. Gestational hypertension
5. Chronic hypertension
6. Seizures in pregnancy
7. Infections
8. Bacterial infections
9. Viral infections
10. *Chlamydophila pneumoniae*
11. *Helicobacter pylori*
12. Cytomegalovirus

Appendix C: Terminology used for PUBMED search engine

Preeclampsia[majr] OR eclampsia OR pregnancy induced hypertension OR Gestational hypertension AND (Bacteria[mh] OR bacterial infections[mh] OR viruses[mh] OR virus diseases[mh] OR Helicobacter pylori[ti] OR chlamydophila pneumonia[ti] OR cytomegalovirus*[ti] OR Infection[ti] OR infections[ti] OR infectious[ti] OR infect[ti] OR infected[ti])

Appendix D: Risk of Bias assessment

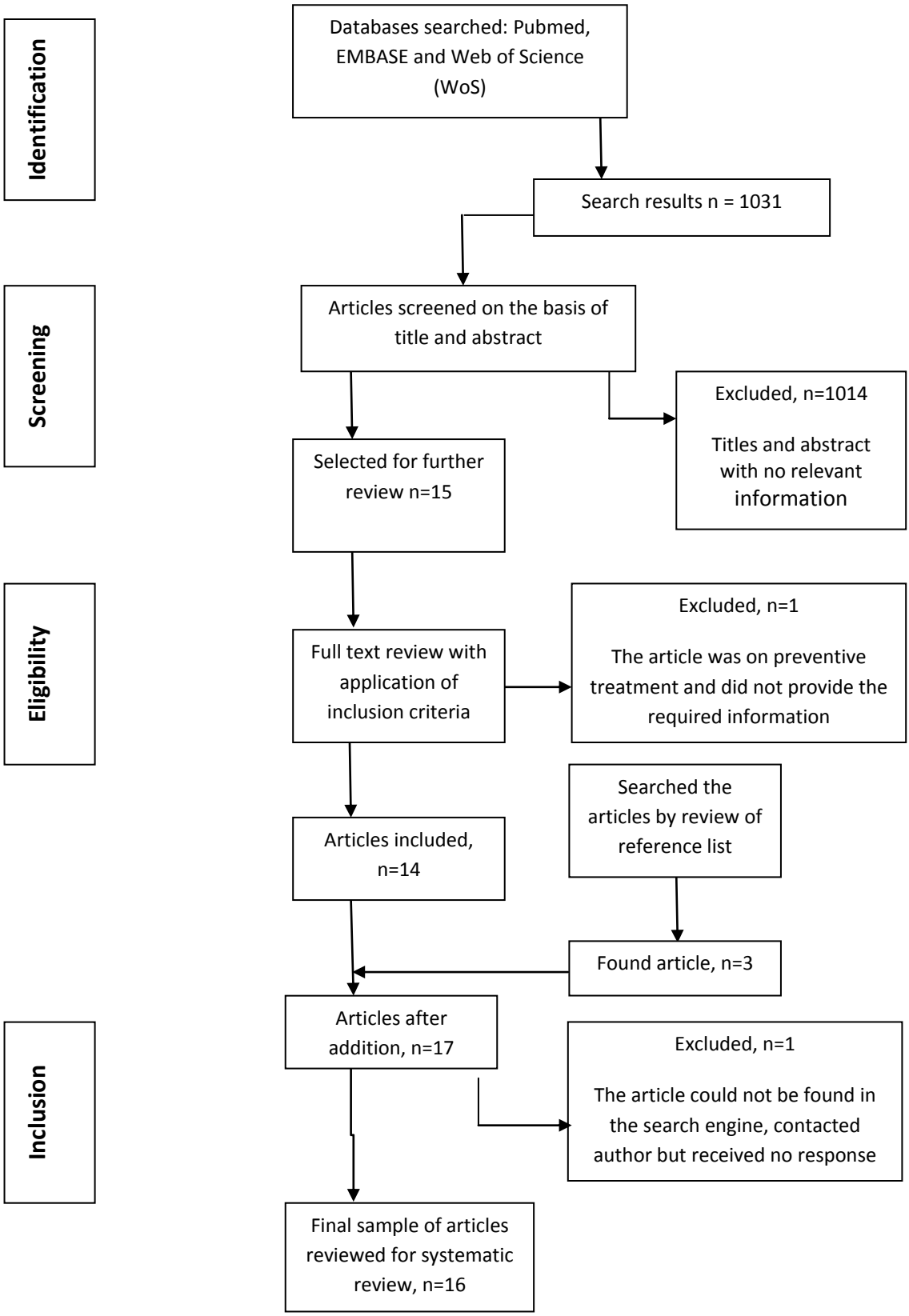
Table 5: Modified version of the ACROBAT-NRSI.

SNo.	Author, Year, Country, Ref	Domains							Assessment of Risk of Bias	Comments
		Bias due to confounding	Bias in selection of participants	Bias in measurement of exposure	Bias due to departures from intended exposure	Bias due to missing data	Bias in measurement of outcome	Bias in selection of reported result		
1	Cardaropoli et al, 2011, Italy (26)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**	Controlled for variables
2	Aksoy et al, 2009, Turkey (37)	Serious risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Serious risk**	
3	Strand et al, 2012, Norway (29)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**	
4	Xie et al, 2010, Canada (27)	Serious risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Serious risk**	
5	Chrisoulidou et al, 2011, UK (36)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**	Matching on variables
6	Karinen et al, 2008, Finland (28)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**	Controlled for variables
7	Aral et al, 2006, Turkey (33)	Critical risk	Moderate risk	Low risk	Low risk	Low risk	Moderate risk	Low risk	**Critical risk**	
8	Goulis et al, 2005, UK (42)	Critical risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Critical risk**	
9	Raynor et al, 2004, USA (43)	Critical risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Critical risk**	
10	Heine et al, 2003, USA (44)	Serious risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Serious risk**	
11	Ustun et al, 2010, Turkey (30)	Critical risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Critical risk**	
12	Von Dadelszen et al, 2003, Canada (45)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**	Matching on variables
13	Ponzetto, 2006, Italy (38)	Moderate risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Moderate risk**	Matching on 2 variables
14	Carreiras et al, 2002, Venezuela (39)	Serious risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Serious risk**	
15	Xie et al, 2010, Canada (40)	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Low risk**	Matching on variables
16	Gomez et al, 2009, USA (41)	Serious risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	**Serious risk**	

ACROBAT-NRSI=A Cochrane Risk of Bias Assessment Tool for Non-Randomized Studies of Interventions.

Appendix E: Search and selection process

Figure 3: Search and selection process of studies



Appendix F: Antigens of H. pylori

Table 6: Types of Antigens of *H. pylori*

Cag	Cytotoxin-associated protein
Vac	Vacuolating cytotoxin protein
UreA	Urease subunits
UreB	
UreC	
HspA	Heat-shock protein
HspB	
Flagellin subunits	
Catalase	
lipopolysaccharide	

Appendix G: PRISMA checklist

Table 7: PRISMA Checklist of items to include when reporting a systematic review (with or without meta-analysis).

Section/Topic	S.No.	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title page, i
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	12
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	14
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	The protocol was not registered
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	15
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	15
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	15 &37
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	15 & 39
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	15-16
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	37

Table 7 (cont'd)

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	27 & 38
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	24-26
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Pooling of results was not done
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Pooling of results was not done
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Pooling of results was not done
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	17 & 39
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	17-19
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	27 & 38
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	21-27
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	28-29 Meta-analysis was not done.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pooling of results was not
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not done.
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	28-29
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	30
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	30

Table 7 (cont'd)

FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	No funding source

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REFERENCES

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