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ORIGINAL ARTICLE



C-reactive protein, interleukin-6 and pre-eclampsia: large-scale evidence from the GenPE case-control study

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ABSTRACT

Multiple small studies have suggested that women with pre-eclampsia present elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6). However, little is known regarding the source of this CRP and IL-6 increase. Therefore, the aim of this study was to evaluate the relationship between CRP and IL-6 levels with pre-eclampsia considering different confounding factors. Using data from a large Colombian case-control study (3,590 cases of pre-eclampsia and 4,564 normotensive controls), CRP and IL-6 levels were measured in 914 cases and 1297 controls. The association between maternal serum levels of CRP and IL-6 with pre-eclampsia risk was evaluated using adjusted logistic regression models. Pre-eclampsia was defined as presence of blood pressure $\geq 140/90$ mmHg and proteinuria ≥ 300 mg/24 h (or $\geq 1+$ dipstick). There was no evidence of association between high levels of CRP and IL-6 with pre-eclampsia after adjusting for the following factors: maternal and gestational age, ethnicity, place and year of recruitment, multiple-pregnancy, socio-economic position, smoking, and presence of infections during pregnancy. The adjusted OR for 1SD increase in log-CRP and log-IL-6 was 0.96 (95%CI 0.85, 1.08) and 1.09 (95%CI 0.97, 1.22), respectively. Although previous reports have suggested an association between high CRP and IL-6 levels with pre-eclampsia, sample size may lack the sufficient power to draw robust conclusions, and this association is likely to be explained by unaccounted biases. Our results, the largest case-control study reported up to date, demonstrate that there is not a causal association between elevated levels of CRP and IL-6 and the presence of pre-eclampsia.

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Pre-eclampsia; inflammation; interleukin-6; C-reactive protein; Colombia; case-control studies

Introduction

Pre-eclampsia is a potentially fatal disease that may appear during pregnancy. In high income countries, around 3% to 5% of the primiparous women develop pre-eclampsia [1]; however, its incidence may be increased up to three times in some low- and middle-income countries [2], becoming the second leading cause of preterm birth and a major risk factor for perinatal and maternal morbidity and mortality [3–4]. In fact, 99% of maternal deaths produced by pregnancy and childbirth complications are originated in low and middle-income nations [5], and it accounts for a third of a million maternal deaths in these countries [6]. Pre-eclampsia has become a health burden for pregnant women in developing countries.

The exact aetiological mechanism of pre-eclampsia remains elusive, and there is no currently effective strategies to prevent the disease or accurately predict women at high risk [7,8]. Recent evidence has led to consider pre-eclampsia

as a cardiovascular disorder (CVD) [9–12] since not only women that suffered it have shown a two-fold increase risk in getting coronary heart disease and stroke in adult life [9,13], but also pre-eclampsia shares several risk factors with CVD, including chronic high blood pressure, dyslipidaemia, obesity and type-2 diabetes [10–14]. In particular, some studies have suggested that CVD risk factors, such as interleukin-6 (IL-6) and C-reactive protein (CRP), might be used as risk factors for pre-eclampsia, as well [15,16].

During pregnancy, both local placental inflammation and a mild systemic inflammatory response are necessary for an adequate placentation [7], which are indicated by high levels of pro-inflammatory cytokines in maternal serum [8,17–20]. Nevertheless, in normal pregnancy, anti-inflammatory molecules are simultaneously produced in order to maintain a balance of this process [21,22]. In pre-eclampsia, an incorrect invasion of trophoblasts leads to placental ischemia [23], increasing the release of inflammatory cytokines, such

as IL-6, that contributes to systemic inflammation and endothelial dysfunction [1,24,25]. Altered production of IL-6 has been associated with the expression of intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM1), providing an adhesive phenotype to the endothelium that leads to its damage [26]. In addition, IL-6 stimulates the production of CRP by the liver, a possible marker of tissue damage and inflammation [27]. Several reports have suggested a negative role of these factors with adverse pregnancy outcomes [28,29].

After decades of research, the IL-6 and CRP role on the etiology of pre-eclampsia is still controversial, and literature presents inconsistent data although the majority of the research reveals a causal relationship between them and pre-eclampsia. Particularly, a systematic review suggests that the pooled weighted mean difference (WMD) in women with higher levels of CRP may have an increased risk of developing pre-eclampsia [30]. Also, a recent systematic review recommends to conduct additional studies to clarify the relevance of IL-6, IL-8 and CRP as consistent predictors of pre-eclampsia [20]. Nevertheless, several authors have found no association of IL-6 and CRP with this disorder. These conflicting data may be explained by several factors. First, current evidence is mostly derived from small-sample-size studies, raising the possibility of small-study bias, particularly publication bias. Secondly, inadequate control for confounding factors might be a major limitation given that 'unadjusted' biomarker differences between cases and controls is the preferred type of analysis. The latter is of significant relevance since women with high blood pressure, LDL-cholesterol, body mass index, type-2 diabetes, and physical inactivity tend to have elevated levels of CRP and IL-6 as well as higher risk of pre-eclampsia [31].

Despite these limitations, a number of inflammatory cytokines, including IL-6, TNF α and CRP, have been reported elevated in women with severe pre-eclampsia, implying both a causal role and a mediator of some clinical manifestations of the disorder [7]. In this context, anti-inflammatory agents may be crucial in not only the management of women with pre-eclampsia but also its prevention [22]. As a result, CRP and IL-6 molecules may be potential therapeutic targets of pre-eclampsia prevention and interventions that modify their maternal serum concentration, only if their causal role is proved [32]. In the present study, we evaluated the possible associations of CRP and IL-6 levels and pre-eclampsia, using the largest sample reported up to date, including almost ten times more cases than any previous studies, and adjusting for confounding factors.

Methods

Study design and participants

GenPE (Genetics and Pre-eclampsia) is a multi-centre case-control study conceived to investigate genetic and non-genetic risk factors for pre-eclampsia [33]. In eight Colombian cities between December 2000 and February 2012, more than 8,000 primigravid women younger than 26 were recruited with and without pre-clampsia at the time of

delivery. We exclude women with history of chronic diseases prior to pregnancy, such as hypertension, kidney disease, diabetes mellitus type II or autoimmune diseases.

Pre-eclampsia was defined as the development of hypertension and presence of proteinuria at or after the 20th week of pregnancy. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, recorded on two consecutive measurements within a 24-hour period. Blood pressure was measured after a five-minute period of rest in a sitting position using mercury sphygmomanometers or electronic devices calibrated against a mercury standard. Proteinuria was defined as measurements above 300 mg in a 24 h collection of urine. If a 24 h collection was not available, proteinuria was defined as $\geq 1+$ on dipstick testing in a random clean-catch mid-stream urine specimen [34]. At least one control was recruited from the same center that provided the case within 1 week (aiming for 24 h) of the case identification. A control was defined as a woman with an uneventful pregnancy in labour at term delivery (37–42 weeks). To improve the homogeneity of the phenotype under evaluation, women with a prior history of autoimmune, metabolic (including diabetes or gestational diabetes), renal, or cardiac (including chronic hypertension) diseases were excluded from the study. In addition, all cases and controls were validated by an outcome committee composed by epidemiologists and consultant obstetricians to further minimize outcome misclassification.

Data collection

A verbal interview with a structured questionnaire in Spanish was conducted by trained personnel at the time of recruitment to ascertain maternal and pregnancy related characteristics. Socio-economic position was recorded in seven categories that are established by Colombian Governmental Classification according to the availability of utilities and features of the household: categories zero to two represent low socio-economic position; categories three and four represent middle socio-economic position; categories five and six represent high socio-economic position. Infections, including vaginosis, urine track infections, sexual transmitted diseases, and others, were recorded as positive if the woman reported at least one event at any moment during pregnancy. Smoking during pregnancy was recorded as positive if a woman had smoked any quantity of any tobacco product at any time during the index pregnancy, disregarding of duration. All participants signed a written informed consent form at recruitment including the use of their information for derived projects from GenPE study.

From the 8,154 women recruited by February 2012 (3,590 cases and 4,564 controls), a total of 2,265 participants had biomarker information for CRP or IL-6 levels. After exclusion of 43 women who were older than 25 years or had missing data for maternal age, gestational age, or ethnicity, the current report includes information from 2,211 unrelated young pregnant women (914 cases and 1,297 healthy pregnant controls).

Several questions were added to the baseline questionnaire during 2004 and 2005 in order to collect data that was not previously recorded regarding maternal weight at the beginning and the end of pregnancy. As a result, pregnancy-weight information was only available for 370 cases and 430 controls that had measurements for at least one of the inflammation biomarkers.

Measurement of inflammatory biomarkers

Blood samples were obtained from the antecubital vein during labour or postpartum, and serum was separated by centrifugation and stored at -80°C until analyses were performed. Measurements of CRP and IL-6 were conducted by a laboratory technician who was blinded to the case-control status. In addition, serum samples of cases and controls were randomly distributed across plates, and the codes assigned to the samples were consecutive; therefore, it was not possible to discriminate between cases and controls based on the codes.

Serum concentrations of IL-6 and CRP were determined using a commercial chemiluminescent immunometric assay (IMMULITE® 2000, DPC, Los Angeles USA) with analytical sensitivity of 2.0 pg/mL for IL-6 and 0.1 mg/L for CRP. The reportable range for CRP was 0.3 to 100 mg/L standardized to WHO 1st IS 85/506. Samples were processed in two batches for CRP and IL-6, without evidence of a difference between them.

Serum concentrations of CRP were available for 899 cases and 1290 controls, while IL-6 concentrations were measured for 844 cases and 1204 controls. Thus, a total of 829 cases and 1197 controls had both biomarkers measured. As an internal quality control, 65 randomly selected samples (35 cases and 30 controls) were run in duplicate. The intra-class correlation for CRP and IL-6 was 0.92 and 0.88 in controls, and 0.73 and 0.78 in cases, respectively.

Ethics approval

Informed consent was obtained from each participant, and ethics approval was granted by the Ethics Review Board of Universidad Autonoma de Bucaramanga in Colombia, for the study protocol before conducting interviews and biochemical analyses (Acta No 0037/2007).

Statistical analysis

Non-normally distributed variables were reported as median and inter-quartile range. Depending of its validity, unpaired Student's t , χ^2 , or Mann-Whitney tests were used to assess the maternal characteristic differences between groups.

CRP and IL-6 were log-transformed to approximate a normal distribution before their introduction in multivariable regression models. Odds ratios (OR) with 95% confidence intervals (CI) were obtained with the use of logistic regression models to estimate the association between maternal concentrations of CRP and IL-6 and pre-eclampsia. The shape of the dose response association between the

log-transformed biomarkers and pre-eclampsia was determined using restricted cubic spline functions after adjusting for maternal and gestational age, recruitment center, and ethnicity. A spline function is a statistical method for modeling nonlinear relationships in which the observed data is left to determine the form of the association between exposure and outcome. For CRP and IL-6, the spline functions were fitted with three internal knots as this provided the best fit according to the Akaike and Bayesian information criteria [35,36]. Departures from linearity were assessed by likelihood-ratio tests (LRT) comparing models with and without the nonlinear components of the splines [37].

A final type of analysis included the biomarkers as continuous variables after log-transformation with one unit corresponding to 1SD of the distribution. The models progressed from unadjusted to adjusted for *a priori* consideration of the following variables as potential confounders: maternal age (years), gestational age (weeks), multiple pregnancies (Yes vs. No), ethnicity (White, Afro-Caribbean, Amerindian, or Mixed), year of recruitment (year), place of recruitment (Bucaramanga/Cucuta, Cartagena, Cucuta, Bogota/Tunja, Medellin, or Cali), socioeconomic position (Low vs. Middle and High), infections during pregnancy (Yes vs. No), and smoking status during pregnancy (Ever vs. Never).

The associations were also tested after adjusting for pre-pregnancy weight (in Kg) in the subsample with available information. All statistical analyses were conducted using STATA version 14 (Stata Corporation, College Station, Texas, United States).

Results

Clinical and demographic characteristics of the participants are reported in Table 1. Gestational age at delivery was on average three weeks earlier in cases than controls, which correlated with both the average lower neonatal weight and higher proportion of newborns with low Apgar score for the cases. Cases also had a higher proportion of history of pre-eclampsia in either mother or sister, and a lower proportion of smokers compared to controls (Table 1). No difference was found between mode of delivery CPR and IL-6 levels and delivery mode so this variable was not included in the final association model (Supplementary material).

The correlation coefficient for log-CRP and log-IL-6 in the control group was $r = 0.291$ ($p < .001$). Table 2 presents the association of maternal risk factors and CRP and IL-6 concentrations in the control group. Women with low socio-economic position and those that self-reported infections during pregnancy had higher levels of CRP and IL-6. In contrast, women that self-report smoking during pregnancy had lower levels of CRP and IL-6. No other association was clearly observed.

The spline models that introduced CRP and IL-6 as continuous variables (in the log-scale) showed a flat association with pre-eclampsia across the range of CRP levels in the GenPE study. The association of maternal serum concentrations of IL-6 with pre-eclampsia appeared to be

circumscribed to high levels of IL-6, although the uncertainty around the estimate was considerable, due to its location at the tail of any distribution, which is compatible with a null association (Figure 1). In the spline models, the LRT showed no departures from linearity ($p > .500$ for both biomarkers); therefore, the associations were summarized with ORs and 95%CI for 1 standard deviation (SD) change in log-transformed values.

After adjusting for maternal and gestational age, place and year of recruitment, multiple pregnancy, socio-economic position, smoking, and infections during pregnancy, the OR for 1SD increase of log-CRP was 0.96 (95%CI 0.85, 1.08). The fully-adjusted OR for 1SD increase of log-IL-6 was 1.09 (95%CI 0.97, 1.22).

Additional adjustment for pre-pregnancy weight in the sub-sample of women with available information did not

alter the results on the evaluated inflammatory biomarkers (data not shown).

Discussion

Main findings

Results from the largest case-control study conducted up to date, including almost 1000 cases, provided no evidence for a substantial increase in the risk of developing pre-eclampsia in young primigravid women in association with higher serum levels of CRP and IL-6.

Strengths and limitations

There are some limitations of the present report that merit careful consideration. Due to the retrospective nature of our study, it is possible that some women with co-existing high blood-pressure and renal disease rather than *de-novo* pre-eclampsia might be misclassified as cases. However, we consider this proportion to be extremely small. First, our definition of pre-eclampsia is standard and uniformly applied in all the participating health centers, and all cases in our study are validated by an outcome committee. Second, the prevalence of high-blood pressure and renal disease in Colombian women with similar age to the cases recruited in GenPE (~20 years old) is extremely low (~0.4%), which further reduces this problem [38].

In this study, quantification of proteinuria was made by 24 h urine or dipstick testing if collection method was not available; in some cases, urine recollection could not be completed due to clinical decision of pregnancy termination for maternal or fetal critical conditions. Although a dipstick reading can be unspecific, this method was also assessed in context with clinical signs and symptoms at recruitment site and then at coordination Centre by a team of physicians and methodologist for double verification of case/control status, so the probability of a case misclassification was low (<5%) [39].

Measurement of both biomarkers used high-sensitivity assays, and was conducted in a blinded fashion to minimize possible differential misclassification. Nevertheless, a

Table 1. Maternal and neonatal characteristics of the study sample.

Characteristics of women	Cases (n = 914)	Controls (n = 1,297)	p Value
Age (years), mean [SD]	19.2 [3.0]	18.8 [2.7]	<.001
Ethnic background, n (%)			
White-Hispanic	90 (9.1)	188 (14.5)	
Afro-Caribbean	125 (13.5)	169 (13.0)	.010
Amerindians	11 (1.2)	11 (0.8)	
Mixed	688 (75.4)	929 (71.3)	
Low socio-economic position, n (%)	787 (86.9)	1108 (86.6)	<.001
PE history in mother, n (%)	137 (15.2)	88 (6.8)	<.001
PE history in any sister*, n (%)	84 (8.6)	62 (4.5)	<.001
C-reactive protein (mg/L), mean [SD]	43.0 [39.9]	41.6 [38.8]	.5760
Interleukin-6 (pg/ml), mean [SD]	46.2 [129.6]	39.9 [106.3]	.3679
From pregnancy			
Gestational age (weeks), mean [SD]	37.0 [3.4]	39.3 [1.2]	<.001
Systolic BP (mm Hg), mean [SD]	150.8 [14.1]	112.2 [9.2]	<.001
Diastolic BP (mm Hg), mean [SD]	100.6 [10.5]	69.0 [8.2]	<.001
Multiple pregnancy, n (%)	29 (3.1)	4 (0.3)	<.001
Smoking in pregnancy, n (%)	13 (1.4)	44 (3.4)	.01
Infections in pregnancy [§] , n (%)	628 (69.2)	864 (66.8)	.06
From newborn			
Male newborn [¶] , n (%)	492 (53.9)	636 (50.2)	.100
Neonatal weight (g)*, mean [SD]	2681.2 [735.4]	3118.2 [422.2]	<.001
Apgar score ≤7 1st minute [¶] , n (%)	235 (25.3)	225 (17.4)	<.001
Apgar score ≤7 5th minute [¶] , n (%)	79 (8.5)	24 (1.9)	<.001

*Calculated among 681 cases and 943 controls that reported having at least one sister who had been pregnant in at least one occasion.

[§]30 cases and 27 controls were excluded for 'don't know' responses.

[¶]Excludes multiple pregnancies.

BP: Blood pressure; PE: pre-eclampsia.

Table 2. Association between maternal risk factors and biomarker concentrations*.

Risk factor	CRP (mg/L)			IL-6 (pg/mL)		
	N controls	Effect (95%CI)	p-value	N controls	Effect (95%CI)	p-value
Maternal age (years)*	1290	-0.01 (-0.08, 0.05)	.682	1204	-0.001 (-0.06, 0.06)	.971
Low socio-economic position	1272	0.34 (0.18, 0.50)	<.001	1190	0.13 (-0.03, 0.30)	.111
PE history in mother [¶]	1054	-0.09 (-0.31, 0.12)	.400	983	0.09 (-0.13, 0.31)	.403
PE history in sister [§]	868	-0.09 (-0.33, 0.16)	.479	819	0.03 (-0.22, 0.28)	.826
Multiple pregnancy	1290	-1.16 (-2.13, -0.18)	.020	1204	0.20 (-0.75, 1.15)	.680
Gestational age (weeks)	1290	0.05 (-0.07, 0.17)	.427	1204	0.07 (-0.06, 0.19)	.320
Smoking in pregnancy	1285	-0.41 (-0.71, -0.11)	.007	1199	-0.43 (-0.72, -0.13)	.004
Infection in pregnancy	1266	0.43 (0.31, 0.54)	<.001	1201	0.30 (0.18, 0.41)	<.001
Weight at beginning pregnancy (kg)	425	0.10 (-0.01, 0.21)	.073	412	-0.06 (-0.16, 0.04)	.231
Weight at time of delivery (kg)	421	0.06 (-0.04, 0.16)	.232	408	-0.01 (-0.10, 0.08)	.768

*Values represent regression coefficients (95% confidence interval) on 1SD of log-transformed biomarkers. For continuous variables, the estimates represent the change in biomarker per increase 1SD of the risk factor. For categorical variables, the estimates represent the difference in biomarker concentration with the reference group.

[¶]Excludes 'don't know' answers.

[§]Excludes 'don't know' answers and women with nulliparous sisters.

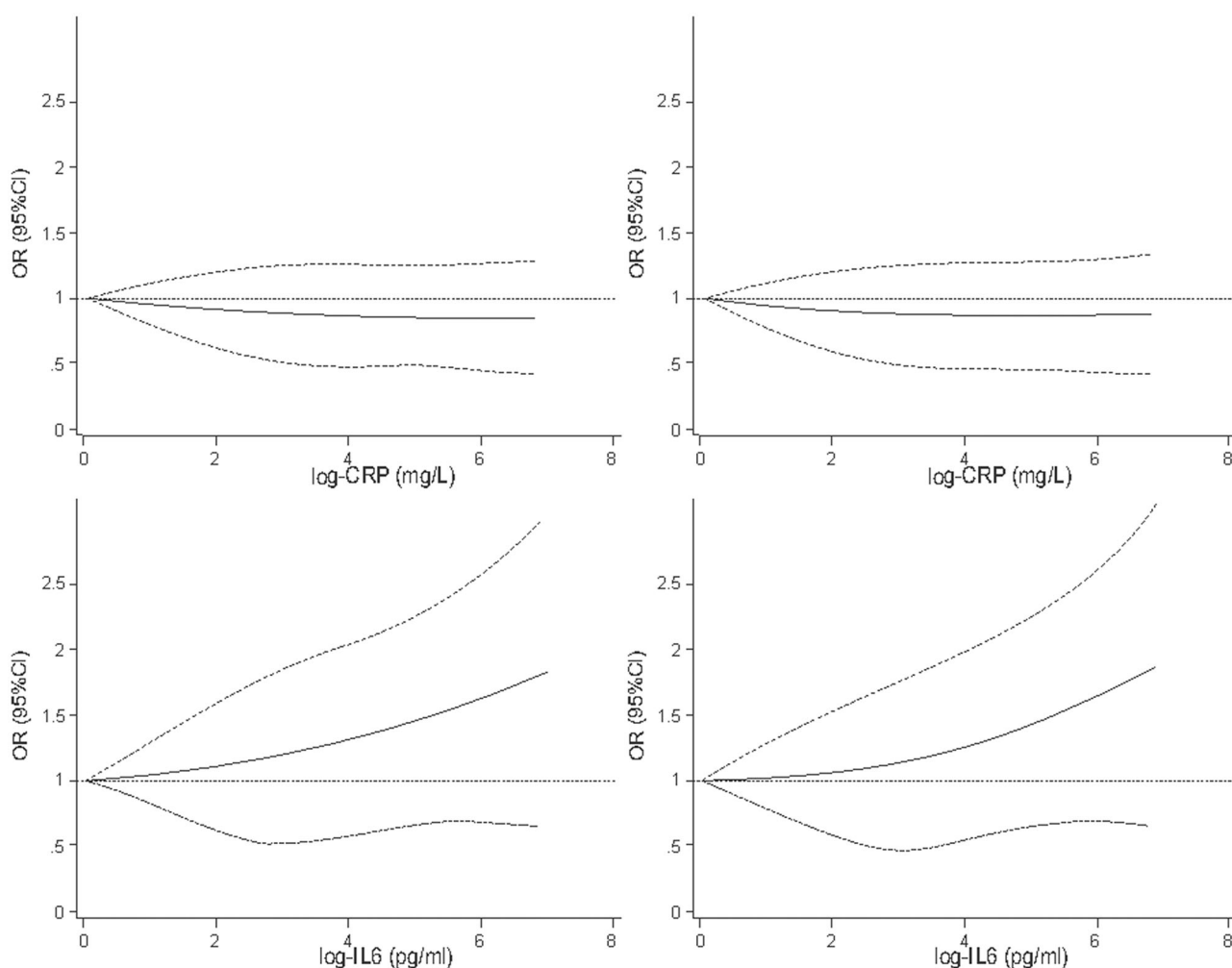


Figure 1. Estimated associations between maternal levels of CRP and IL-6 on pre-eclampsia risk using different degrees of adjustment. This figure presents smooth curves for odds ratios for pre-eclampsia according to maternal levels of CRP and IL-6 after progressive adjustment. The curves were derived from spline models including the biomarker levels after logarithmic transformation. Both axes are presented in a log-scale. Continuous lines indicate OR. Dashed lines indicate 95% Confidence Intervals. Minimal adjustment: maternal age, gestational age, recruitment centre and ethnicity. Full adjustment: minimal adjustment plus recruitment year, multiple pregnancy, smoking, socioeconomic status and infections during pregnancy.

limitation of our study includes the measurement of CRP and IL-6 levels in late pregnancy, which may lead to reverse causation bias. In this way, an additional concern related to CRP levels is the mode of delivery because c-section could have a stronger inflammatory response; nonetheless, it has been reported that only duration of labor was associated with higher levels [Geometric Means Ratio 1.15 (IC95% 1.04; 1.27)], although association strength was small [40].

Although it is theoretically possible that the effect of CRP and/or IL-6 is more specific to certain type of population different to the one we evaluated, we consider this scenario as unlikely. Several of the published studies reporting an association of high levels of CRP and IL-6 with pre-eclampsia were only conducted in primigravid women from Latin America, similar to our study [41–43]. In addition, previous evidence have indicated that patterns of risk factors among Latin American and Caribbean women (comparable to the women included in the GenPE study) are similar to those reported in North America and European women [44].

There are several lines of evidence that support the lack of association of CRP and pre-eclampsia observed in the current study. Similar null results were also found in a nested case-control study based on the calcium for pre-eclampsia prevention trial in North America, the second largest study (after this report) [45]. In contrast, most of the evidence supporting the association of CRP with preeclampsia derives from very small studies, which is demonstrated by the latest systematic review published in 2013 in which 21 out of the 23 studies evaluated included less than 100 cases [30].

Discrepancies between large and small studies are not new in observational reports and may be due to multiple causes such as genuine heterogeneity, chance findings, undue emphasis on sub-group analyses, ‘significance chasing,’ or publication bias [46]. Another important reason that may explain the discrepancy between our case-control study and positive published studies is the lack of adequate control for confounding when evaluating the CRP-pre-eclampsia association. An example of this is demonstrated by

another recent systematic review in which only a minority of the published studies identified reported adjusted measures of associations derived from multiple regression models [47].

Inadequate control of confounding could distort the observed association of CRP with pre-eclampsia. The socio-economic pattern in CRP distribution observed in our study, and previously reported by others [48,49], as well as the established associations of CRP with other risk factors for preeclampsia [31] makes confounding an important possibility to account for when estimating a difference in maternal levels between women with pre-eclampsia and healthy pregnant controls.

The results from observational studies should be also interpreted in the context of other lines of evidence. Findings from earlier mechanistic studies on the biological effects attributed to CRP of relevance to pre-eclampsia (endothelial dysfunction, impairment in vascular reactivity and increase in blood pressure) [31] have not been replicated by rigorous and controlled studies [38]. It has been suggested that the effects of CRP observed in the earlier experimental studies could have been affected by the presence of pro-inflammatory substances contaminating commercially-sourced recombinant CRP from bacterial sources, or to the preservative sodium azide used in all commercial preparations as a bacteriostatic [31]. Altogether, these different lines of evidence strongly discourage CRP as a causal factor as well as therapeutic target in pre-eclampsia.

Although our findings on IL-6 failed to confirm a substantial increase in the risk of pre-eclampsia (confidence intervals are still wide and compatible with a null association), it is important to note that the spline model suggested a potential dose-response association. Therefore, we cannot conclusively reject the presence of a small to moderate association of circulating levels of IL-6 with pre-eclampsia. Just like with CRP, results from published studies on IL-6 and pre-eclampsia are also affected by small-study bias and inadequate control for confounding; thus, it is not surprising that evidence for this association is largely inconsistent.

It is important to note, that our findings, however, do not exclude a role for the systemic or local inflammatory response in pre-eclampsia. The effects of other markers of the inflammatory response might still be important in pre-eclampsia.

Conclusion

Evidence from our study argues against an important role of CRP and IL-6 in pre-eclampsia. The most likely explanations for the apparent association of these biomarkers with pre-eclampsia from published studies are small-study bias and inadequate control for confounding. The current findings, however, do not exclude a role of inflammation in pre-eclampsia development, but highlight the imperative need to identify the specific biomarkers responsible for this association in order to generate specific interventions that can modify the risk of pre-eclampsia. Due to the higher

incidence and burden of pre-eclampsia in low- and middle-income countries, it will be more cost effective to generate such resources in those settings, while maintaining quality standards. This study is an example of high-quality international observational studies conducted in low- and middle-income settings that showed that this alternative is now viable.

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Contribution to authorship

All authors significantly contributed to the intellectual content of this manuscript during the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting or revising the content, and final approval of the paper. NCS and JPC designed the study, and MB, CCM, PKBN, SES, RO, SMB, AM, JM, CMM, MLL, and WS, enrolled patients, collected data, and systematically reviewed the relevant literature. CCM, MCP, and EG were involved in data transfer and cleaning. DCQL analyzed the data, with support from CCM, JPC, and NCS. NCS and JPC were involved in data interpretation and wrote the first draft of the manuscript, which was then reviewed and revised by all co-authors.

Disclosure statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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