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HELICOBACTER PYLORI COLONIZATION AND PREGNANCIES COMPLICATED BY PREECLAMPSIA, SPONTANEOUS PREMATURITY AND SMALL FOR GESTATIONAL AGE BIRTH

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Abstract

Background—Preeclampsia (PE), small for gestational age (SGA), and spontaneous preterm birth (PTB) each may be complications of impaired placental function in pregnancy. Although their exact pathogenesis is still unknown, certain infectious agents seem to play a role. *Helicobacter pylori (H. pylori)* colonization has been associated with increased risk for PE. Our aim was to assess the association between *H. pylori* colonization and PE, SGA, and PTB.

Material and methods—We measured IgG anti-*H. pylori* and CagA-antibodies in serum of pregnant women (median 20.5 weeks, range 16.5–29.4) who participated in a population-based prospective cohort study. Delivery and medical records were assessed. Information on demographics, education, and maternal risk factors was collected by questionnaire. We used multivariate logistic regression analyses to assess associations between *H. pylori* colonization and PE, SGA, and PTB.

Results—In total, 6348 pregnant women were assessed. *H. pylori*-positivity was found in 2915 (46%) women, of whom 1023 (35%) also were CagA-positive. Pregnancy was complicated by PE, SGA or PTB in 927 (15%) women. *H. pylori* colonization was associated with PE (aOR 1.51; 95%CI 1.03–2.25). Differentiation according to CagA-status revealed the same risk. *H. pylori* was

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positively related with SGA, mainly explained by CagA-positive strains (aOR 1.34; 1.04–1.71). No association was observed between *H. pylori* and PTB.

Conclusions—Our data suggest that *H. pylori* colonization may be a risk factor for PE and SGA. If these associations are confirmed by future studies and shown to be causal, *H. pylori* eradication may reduce related perinatal morbidity and mortality.

Keywords

H. pylori colonization; virulence factor CagA; preeclampsia; small for gestational age; spontaneous preterm birth

INTRODUCTION

The involvement of systemic inflammatory responses in pregnancies complicated by pre-eclampsia (PE), small for gestational age (SGA), and spontaneous preterm birth (PTB) has led to the hypothesis that maternal infections may play a role in the etiology and pathogenesis of these pregnancy complications (1, 2). Although the exact causes of these complications are still unknown, one hypothesis for their origin is that they each are related to suboptimal placentation in early pregnancy (3–5). In this respect, colonization with *Helicobacter pylori (H. pylori)* may be of interest as it might be involved in the pathogenesis of impaired trophoblast invasiveness (6).

H. pylori is a Gram-negative bacterium that colonizes the stomach of about half of the world's population. After its re-discovery in 1982, extensive research demonstrated that *H. pylori* is an important risk factor for peptic ulcer disease, gastric adenocarcinoma, and mucosa associated lymphoid tissue (MALT)-lymphoma (7). An important host-interaction factor of *H. pylori* is the cytotoxin-associated gene A (cagA). The CagA protein is directly injected by *H. pylori* into the cytoplasm of gastric epithelial cells and subsequently affects cell morphology, proliferation and apoptosis (8). Colonization with CagA-positive strains is associated with higher levels of inflammatory cells and mediators compared to CagA-negative strains, both locally and systemically (9).

As such, recent studies have focused on extra-gastric manifestations of *H. pylori* colonization, including cardiovascular, hematologic, respiratory, and pregnancy-related diseases, including PE, SGA, and PTB (10). However, only few studies, each with a small number of cases, assessed the associations between *H. pylori* colonization and PE (11–14), and SGA (12, 15). These studies yielded conflicting results. Therefore, we examined the association between *H. pylori* colonization and each of these pregnancy-related complications in pregnant women participating in a large population-based prospective cohort study. As colonization with a CagA-positive strain is associated with higher levels of inflammatory mediators (16), we also assessed the effects of CagA-positive *H. pylori* strains on the risk of having these illnesses.

MATERIALS AND METHODS

Design and setting

This study was embedded in The Generation R Study, a population-based prospective cohort study among women and their children in Rotterdam, The Netherlands. In total 8879 pregnant women were included between April 2002 and January 2006. Assessments consisted of physical examinations, fetal ultrasounds, biological samples, and questionnaires (17, 18). Approval was obtained from the Medical Ethics Committee of the Erasmus Medical Center. All participants provided written informed consent. *H. pylori* status could be measured in 6837 women. For the present study, women with maternal comorbidity known to be associated with an increased risk for the occurrence of these three illnesses (i.e. chronic hypertension, heart disease, diabetes, high cholesterol, thyroid disease and systemic lupus erythematosus) were excluded (n=179). Twin pregnancies, and women without data on PE, SGA, and PTB were also excluded. This left a study population of 6348 pregnant women with available information on both *H. pylori* status and pregnancy complications (Figure 1).

H. pylori colonization during pregnancy

Mid-pregnancy serum samples (median 20.5 weeks, range 16.5–29.4) were examined for IgG antibodies against *H. pylori* and the cytotoxin-associated gene A (CagA) protein using two separate enzyme-linked immunosorbent assays (ELISA), as described (19, 20). All samples were measured at least in duplicate. For each sample, the optical density ratio (ODR) was calculated by dividing the optical density (OD) by the mean OD of the positive controls. *H. pylori* positivity was defined as either an ODR≥1 or CagA positivity. The cut-off for CagA positivity was an ODR value ≥0.35. Details regarding *H. pylori* colonization in this cohort of pregnant women have been described (21). Both ELISAs were validated locally.

Pregnancy complications: PE, SGA, and PTB

Information on the pregnancy complications PE, SGA and PTB was obtained from medical records. For women who had suspected PE, the records were cross-checked with the original hospital charts (22). PE was defined as the development of systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg after 20 weeks of gestation in previously normotensive women, with concurrent proteinuria (≥0.3 g in a 24-hour urine specimen or ≥2+ [1 g/L] from a voided specimen, or ≥1+ [0.3 g/L] from a catheterized specimen) (23). Pregnancies complicated by hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome (n=22) were defined as cases of preeclampsia (23). PTB was defined as spontaneous onset of birth <37 weeks of gestation. We defined SGA as birth weight under the 10^{th} percentile based on reference curves from our own cohort (24). Birth weight was adjusted for gestational age and transformed resulting in a normal distribution, which allowed use of means and standard deviations (SD) (25).

Covariates

Data on maternal age, ethnicity, educational level, parity, smoking during pregnancy, and maternal comorbidity (chronic hypertension, heart disease, diabetes, high cholesterol, thyroid disease and systemic lupus erythematosus) were obtained from questionnaires repeatedly applied during pregnancy. Height and weight were measured at enrolment and body mass index (BMI, kg/m^2) was calculated. In Rotterdam, the largest ethnic groups are of Dutch, Surinamese, Turkish, Moroccan, Dutch-Antilles, and Cape Verdean descent. Women with another ethnic background were categorized into 'other Western' (European, North American, Oceanean) or 'other non-Western' (African, Asian, South- and Central American). The highest educational level of the mother was defined as completion of university or higher vocational training. Mothers were categorized as having a middle/low level of education if they had completed intermediate vocational training, or had completed education below that level.

Statistical analysis

Firstly, with respect to missing data on BMI (0.6%), ethnicity (6.5%), educational level (8.1%), parity (0.8%) and smoking (11.9%), values were imputed using multiple imputation (26). In the present study, five draws for each missing value were performed providing five substituted data points, which in turn created five completed data sets. Analyses were performed separately on each completed dataset and thereafter combined into one global result (26). Supplementary Table 1 provides the percentages of missing values per covariate. Secondly, the frequency distributions between risk factors for pregnancy complications and *H. pylori* colonization were examined using the Independent Students' t-test (normally distributed continuous data) and the Chi-square test (categorical data). Then, univariate and multivariate logistic regression analyses were applied to assess the associations between *H. pylori* colonization and each separate pregnancy outcome (i.e. PE, PTB, and SGA). A number of cases was diagnosed with more than one of these pregnancy complications. The inclusion of potential confounders was based on earlier literature (4, 27) and/or if they changed the effect estimates with ≥10%. We used three regression models to explore the effect of potential confounders on the association between *H. pylori* and PE, PTB, and SGA. In model 1, we adjusted for maternal age, ethnicity and parity. Model 2 was additionally adjusted for body mass index and smoking during pregnancy. The third model was also adjusted for maternal education level as a proxy for socio-economic status. Analyses regarding *H. pylori* and SGA were also adjusted for fetal gender in all three models. Lastly, to examine effect modification we evaluated statistical interaction. Effect modification occurs when the magnitude of the effect of *H. pylori* on the outcome (PE, SGA, or PTB) differs depending on the level of a third covariate (i.e. maternal educational level, parity, smoking, body mass index, and fetal gender). The latter may be involved with sex specific associations regarding placentation (28). We evaluated this statistical interaction, by adding a new variable to the analysis, which is the product term of *H. pylori* status and the covariate. If the p-value for interaction was <0.05, a stratified analysis according to the specific covariate was performed. All measures of associations are presented as Odds Ratios (OR) with their 95% Confidence Intervals (CI). Statistical analyses were performed using IBM SPSS Statistics 21.0 for Windows (SPSS IBM, Armonk, New York, USA).

RESULTS

Population characteristics

In total, 6348 women were included in the study. Their baseline characteristics are shown in Table 1. Mean age at enrolment was 29.7 years (SD 5.3), and 51.0% were of non-Dutch ethnic background. Overall, the *H. pylori* colonization rate was 45.9%, of whom 35.1% carried CagA-positive strains. Nine-hundred and twenty-seven (14.6%) developed either PE, SGA or PTB. Compared to women without one of the indicator conditions, women with a complicated pregnancy were younger, had attained a lower level of education, were more often of non-Dutch ethnicity, had a lower BMI, did smoke more often during pregnancy, and were more often pregnant with their first child. (Table 1, $p<0.001$ for univariate comparisons; see supplementary table 1 for observed and imputed data). In total, 129 (2.0%) women were diagnosed with PE, 638 (10.1%) with SGA, and 219 (3.4%) with PTB (Figure 1). Fifty-eight women (0.9%) had more than one of these complications, and contribute to both complication groups.

H. pylori colonization and PE, SGA, and PTB

Women with one of these pregnancy complications were more often *H. pylori* positive (51.0%) than women with an uncomplicated pregnancy (45.0%) ($p<0.001$). Among those women with *H. pylori*, the CagA-positivity rate was higher in those with a complicated pregnancy (39.1% vs. 34.3%, $p<0.001$) (Table 1).

Supplementary table 2 shows the prevalence of PE, SGA, and PTB according to *H. pylori* and CagA-status. Univariate logistic regression analyses showed an increased risk of PE and SGA in *H. pylori*-positive mothers (OR 1.49; 95% CI 1.05–2.12 and OR 1.32; 1.12–1.56, respectively) (Supplementary table 3). Parallel results for *H. pylori* and SGA were observed when using an ethnic-specific 10^{th} percentile definition for SGA (data not shown). Differentiation into CagA-negative and CagA-positive strains revealed a positive association between *Hp+*CagA+ mothers and SGA (OR 1.59; 95% CI 1.28–1.97). No association was observed between *H. pylori* and PTB. Multivariate analyses revealed an association with PE (final OR 1.51; 95%CI 1.03–2.25), but not with SGA or PTB (Table 2). Differentiation into CagA-negative or CagA-positive strains showed an association between CagA-positive strains and SGA (final OR 1.34; 95% CI 1.04–1.71). Increased risk of PE was observed in mothers with a CagA-negative strain (Model 2, OR 1.58; 95% CI 1.03–2.40). The association attenuated slightly after additional adjustment for educational level. We did not observe an interaction between *H. pylori* status and fetal gender, maternal educational level, parity, smoking, and body mass index (data not shown).

When excluding cases with more than one pregnancy complication from each group, multivariate analyses showed only CagA-positivity independently associated with SGA (OR 1.33; 95% CI 1.03–1.72) (Supplementary table 4). Multivariate analysis of pregnancies complicated by both PE and SGA (n = 38) showed a trend to increased risk in *H. pylori* positive women (OR 1.71; 95% 0.83–3.53).

DISCUSSION

This large population-based prospective cohort study showed that *H. pylori* colonization is associated with an increased risk on PE and that carriage of a CagA+ *H. pylori* strain is a risk factor for SGA. These findings may be helpful for a better understanding of the pathogenesis of these pregnancy complications and support the link with chronic inflammatory conditions. The potential association between *H. pylori* and these gestational disorders has been studied before. Previous studies however were considerably smaller, thereby limiting power. Furthermore, not all studies included separate analyses of CagA data.

The observed association between *H. pylori* and PE is consistent with prior epidemiological studies (11–14). In a small Italian study of 47 PE cases and 47 controls, Ponzetto et al. found a higher *H. pylori* seropositivity in mothers with PE (51.1%) compared to women with an uncomplicated pregnancy (31.9%) (OR 2.67; 95% CI 1.08–6.57) (11). In contrast to our observation, they observed greater differences between those having CagA-positive and CagA-negative strains (80.9% vs. 14.9%). Another study from the same group investigated the association of several *H. pylori* virulence factors with fetal growth retardation (FGR) (FGR, n = 13), PE (n = 17), and both (PE and FGR, n = 32) compared with controls (n = 49) (12). In PE women with or without FGR, the *H. pylori*-positivity rate was higher while there was no difference in prevalence between FGR-only and controls. They observed that CagA-strains were more prevalent in PE pregnant women compared to controls, while there was no difference between FGR cases and controls. Besides the small number of cases compared to our study, the study differs in case definition as they used FGR and we SGA as proxy for FGR. An Australian study determined the association between *H. pylori* colonization and SGA in 448 pregnant women (15), of whom 34 (7.5%) had SGA. Multivariate analysis revealed that *H. pylori* seropositivity was associated with SGA (OR 2.59; 95% CI 1.12–5.95; p = 0.025). A similar trend was observed in our study, but only for those with CagA-positive strains. CagA status data were not available in the prior study. We found no association between *H. pylori* and PTB, regardless of CagA-status. One other study, assessing this relation in 416 pregnant women, did not observe a significant association between *H. pylori* colonization and PTB (29).

The overall *H. pylori* prevalence in this cohort may be higher than for other populations in a Western country. This difference is mainly explained by the high colonization rates in women with a non-European ethnic background. Studies evaluating the *H. pylori* colonization in multi-ethnic populations all showed higher prevalence among immigrant groups, compared to the original population (30, 31). Although PE, SGA, and PTB are different clinical entities, all three may be caused by suboptimal deep placentation in early pregnancy (5) (32, 33) (34, 35). Large numbers of non-transformed spiral arteries are frequently observed in PE patients with or without SGA (5), in patients with SGA without gestational hypertension (32, 33), and in patients with preterm labor with or without preterm pre-labor rupture of membranes (PROM) (34, 35). Impaired remodeling of the spiral arteries may lead to insufficient uteroplacental arterial flow and episodes of irregular placental perfusion (3). Such impaired remodeling might be due to failure of appropriate uterine preconditioning (36), and excessive or atypical maternal immune responses to trophoblasts

(37). Franceschi et al. have shown that anti-CagA antibodies were able to recognize β-actin on the surface of trophoblast cells in a dose-dependent binding assay *in vitro* (6). This binding resulted in impaired cytotrophoblast invasiveness, which is characteristic for the development of the placental syndrome; however, we observed no association between CagA-positive strains and PE. The association between *H. pylori* and PE disappeared when excluding women with more than one of the studied pregnancy complications (i.e. with both PE and SGA), which suggests that the significant result was based on those cases. PTB is a syndrome caused by multiple pathologic processes with placental involvement as one of the possibilities (38). In our study, although all spontaneous in onset, we were not able to distinguish between different causes of PTB.

Our data add further evidence to the associations between *H. pylori* and PE and SGA. Although association does not imply causation, epidemiological findings should stimulate biological studies. If the association between *H. pylori* and these illnesses is causal, eradication of *H. pylori* may be part of an effective intervention for reducing related perinatal morbidity and mortality. As the overall *H. pylori* prevalence in Western countries is declining, screening for *H. pylori* may be most efficient in pregnant women with increased *H. pylori* prevalence, like a low socioeconomic status, or a non-Western ethnic background (21). Nevertheless, we think that more evidence is needed, before organizing eradication programs in pregnant or non-pregnant women.

The strengths of this study include the large number of subjects participating in a population-based cohort, with detailed prospectively collected data on socioeconomic, and sociodemographic characteristics, together with other potential confounding factors. However, it cannot be excluded that the findings may partly result from unmeasured confounding. Our study may be limited by the fact that we measured IgG antibodies against *H. pylori* and CagA, indicating present or recent colonization. However, it is not clear whether any effect of *H. pylori* is dependent on active colonization or the presence of circulating anti-*H. pylori* and CagA antibodies. The use of one general definition for SGA in all ethnic groups may limit our results, as a prior study has shown differences in birth weights between ethnic populations of this cohort (39). However, additional analyses, using the ethnic-specific 10^{th} percentile revealed parallel results for *H. pylori* and SGA. Since validated ethnic-specific growth curves are lacking, we continued to use the population-specific 10^{th} percentile. Although this study is population-based, selective participation occurred since participating women were generally higher educated, and were more often from Dutch ethnic background (17). Missing data for several characteristics and potential confounders may have biased the outcome. Therefore, we performed the final analyses after a multiple imputation procedure. This is considered useful to deal with missing data, since it requires the fewest assumptions and reduces potential bias when missing data are not random (26).

In summary, *H. pylori* colonization is positively associated with PE. In addition, we confirmed the important role of CagA-positive strains in SGA, as the association was determined by colonization with these strains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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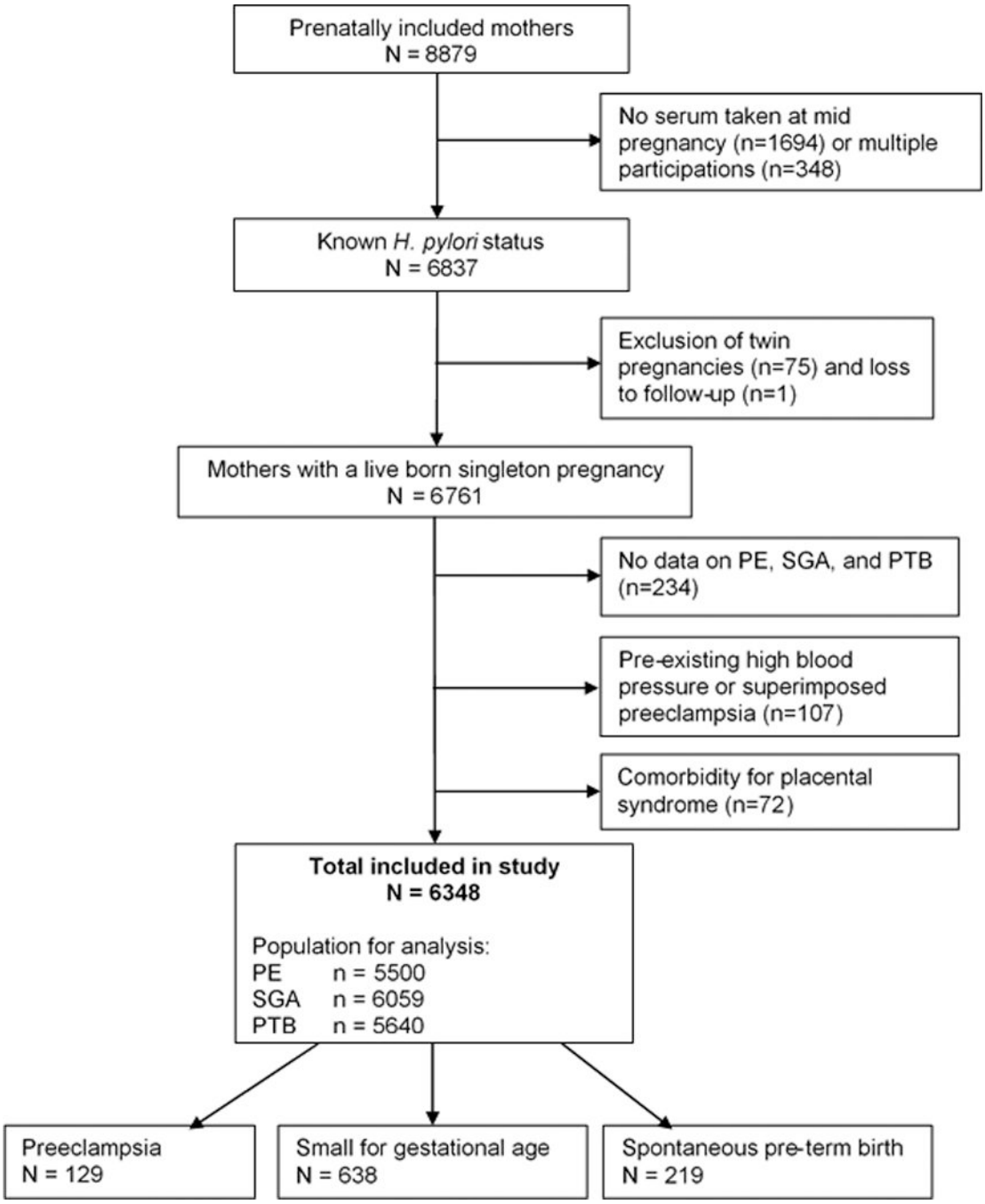

Figure 1.
Study design

Table 1

Characteristics of mothers (n = 6348)

	Imputed	Complicated pregnancy[1]		p-value
		No (n=5421)	Yes (n=927)	
Age, years (SD)	29.7 (5.3)	29.9 (5.2)	28.9 (5.6)	<0.001
Body mass index, kg/m^2 (range)	23.7 (15.2–51.2)	23.8 (15.2–51.2)	23.3 (15.8–50.2)	<0.001
Data missing				
Ethnicity (%)				<0.001
Dutch	3031 (47.7)	2656 (49.0)	375 (40.1)	
Turkish	654 (10.3)	546 (10.1)	88 (9.5)	
Surinamese	569 (9.0)	433 (8.0)	143 (15.4)	
Moroccan	458 (7.2)	421 (7.8)	45 (4.9)	
Cape Verdean	302 (4.8)	235 (4.3)	68 (7.3)	
Dutch Antillean	246 (3.9)	190 (3.5)	59 (6.4)	
Other Western	689 (10.9)	605 (11.2)	86 (9.3)	
Other non-Western	399 (6.3)	335 (6.2)	63 (6.8)	
Data Missing				
Education level (%)				<0.001
Low/Middle	3807 (60.0)	3178 (58.6)	628 (67.7)	
High	2541 (40.0)	2243 (41.4)	299 (32.3)	
Data missing				
Parity (%)				<0.001
Nulli parity	3503 (55.2)	2865 (52.9)	638 (68.8)	
Multi parity	2845 (44.8)	2556 (47.1)	289 (31.2)	
Data missing				
Smoking during pregnancy (%)				<0.001
No	5136 (80.9)	4462 (82.3)	675 (72.8)	
Yes	1211 (19.1)	959 (17.7)	252 (27.2)	
Data missing				
Children's gender				0.65
Female	3209 (50.5)	2733 (50.4)	475 (51.2)	
Male	3139 (49.5)	2687 (49.6)	452 (48.8)	
Data missing				
H. pylori and CagA				
H. pylori-negative	3433 (54.1)	2979 (55.0)	454 (49.0)	<0.001
H. pylori-positive	2915 (45.9)	2442 (45.0)	473 (51.0)	
CagA-negative	1892 (64.9)	1604 (65.7)	288 (60.9)	<0.001
CagA-positive	1023 (35.1)	838 (34.3)	185 (39.1)	

Values are means (SD), medians (range) or absolute numbers (percentages).

[1] Pregnancy complicated by PE, SGA, or PTB

Table 2

Associations of *H. pylori* and CagA status with PE, SGA, and PTB.

	Preeclampsia (n = 5550)			Small for gestational age (n = 6059)			Spontaneous preterm birth (n = 5640)		
Model	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Hp−	*Reference* *n = 58 / 3037*	*Reference*	*Reference*	*Reference* *n = 306 / 3285*	*Reference*	*Reference*	*Reference* *n = 113 / 3092*	*Reference*	*Reference*
Hp+	**1.59** **(1.07, 2.35)*** n = 71 / 2513	**1.57** **(1.06, 2.32)***	**1.51** **(1.03, 2.25)***	1.20 (0.99, 1.46) n = 332 / 2774	1.18 (0.98, 1.43)	1.16 (0.96, 1.41)	1.18 (0.87, 1.61) n = 106 / 2548	1.17 (0.86, 1.60)	1.15 (0.84, 1.57)
Hp+CagA−	**1.58** **(1.04, 2.40)*** n = 46 / 1650	**1.58** **(1.03, 2.40)***	1.53 (1.00, 2.33)	1.12 (0.91, 1.38) n = 195 / 1799	1.09 (0.89, 1.35)	1.08 (0.87, 1.33)	1.21 (0.86, 1.68) n = 70 / 1674	1.19 (0.86, 1.67)	1.18 (0.84, 1.64)
Hp+CagA+	1.61 (0.95, 2.73) n = 25 / 863	1.56 (0.92, 2.64)	1.50 (0.89, 2.55)	**1.38** **(1.08, 1.76)*** n = 137 / 975	**1.36** **(1.06, 1.74)***	**1.34** **(1.04, 1.71)***	1.13 (0.73, 1.73) n = 36 / 874	1.11 (0.72, 1.70)	1.08 (0.71, 1.66)

Values are odds ratios for PE, SGA, and PTB (95% confidence interval) from logistic regression models. n = number of cases per total group.

Model 1 was adjusted for maternal age, parity, ethnicity.
Model 2: model 1 additionally adjusted for body mass index and smoking.
Model 3: model 2 additionally adjusted for educational level.

Analyses regarding *H. pylori* and SGA were also adjusted for fetal gender in all three models.

*p<0.05