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***Helicobacter pylori* seropositivity and pregnancy-related diseases: a prospective cohort study**

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Abbreviations

AGA: Appropriate for Gestational Age

BMI: Body Mass Index

CI: Confidence Interval

FGR: Fetal Growth Restriction

GDM: Gestational Diabetes Mellitus

HELLP: Hemolysis, Elevated Liver enzymes and Low Platelet count

H.pylori: Helicobacter pylori

LGA: *Large for Gestational Age*

OR: Odds Ratio

PE: Pre-eclampsia

PIH: Pregnancy-Induced Hypertension

pPROM: preterm Premature Rupture Of Membranes

SGA: Small for Gestational Age

ABSTRACT

The relationship between *Helicobacter pylori* infection and extragastric disease is well established. This study prospectively investigated whether maternal *H. pylori* seropositivity, detected during the first half of pregnancy, could be associated with the development of the major pregnancy-related pathologies during late second or third trimester in a general population. Our hypothesis was that *H.pylori* infection could negatively influence pregnancy development and outcome. A total of 2,820 consecutive pregnant women were recruited before 20 weeks of gestation, from October 2008 to August 2010, and blood samples were collected from each subject. IgG antibodies against *H.pylori* were assayed in maternal serum by a commercial immunoassay. Logistic regression analyses were performed to assess any association between *H.pylori* seropositivity and adverse pregnancy outcomes. Gestational diabetes mellitus (GDM) was the most common maternal complication (5.7%) and the only pregnancy-related disorder with a significantly higher rate of *H.pylori* positive women (41.3%) compared to subjects who did not develop the disease (27.7%) ($P < 0.001$; OR = 1.829, 95% CI = 1.320 – 2.533). The difference observed remained statistically significant after adjusting for potential confounding variables. The presence of antibodies against *H.pylori* antigens in maternal serum was independently associated with the development of GDM. These findings suggest a potential role for *H.pylori* eradication in the prevention of gestational diabetes mellitus.

Key words: *Helicobacter pylori*; infection; pregnancy outcome; gestational diabetes mellitus.

1. Introduction

Pre-eclampsia (PE), fetal growth restriction (FGR), preterm premature rupture of membranes (pPROM) and gestational diabetes mellitus (GDM) are serious pregnancy-related disorders having adverse effects on both maternal and fetal safety. A reduction in maternal mortality attributable to unfavorable pregnancy outcome has been observed in developed countries. However, perinatal mortality, perinatal and long term morbidity as well as neurological sequelae due to abnormal fetal growth and/or preterm delivery, remain high. Timely, and often preterm, delivery is the only current effective treatment. Nowadays there are only few options open to prevent these disorders, partly due to the fact that the etiology and the pathogenesis of pregnancy-related disorders are still poorly understood. We are of the opinion that infection alone, or as a cofactor in women with genetic susceptibility to adverse pregnancy outcome, might well play a role in the etiology and pathogenesis of pregnancy complications.

Helicobacter pylori (*H.pylori*) infection affects approximately one half of the world's population and is commonly associated with chronic gastritis, which increases the risk of many serious gastrointestinal complications, such as peptic ulcer disease and gastric cancer (Malaty, 2007). This is supported by several reports that indicate a correlation between *H.pylori* infection and various extra-gastric disorders (Banić et al., 2012), including some serious pregnancy-related pathologies, such as pre-eclampsia and/or fetal growth restriction (Cardaropoli et al., 2014). These pregnancy disorders, as well as numerous cases of miscarriage, mainly involve abnormal placentation. Indeed, it has recently demonstrated that antibodies against the *H.pylori* virulence factor CagA cross react *in vitro* with cytotrophoblast cells, reducing their invasive ability (Franceschi et al., 2012).

Most publications on the correlation between *H.pylori* infection and pregnancy-related disorders are cross-sectional investigations. The only prospective study on *H.pylori* infection and pregnancy-related complications reported an association between maternal infections caused by *H.pylori* CagA-strains and early pregnancy loss in patients given intra-cytoplasmic sperm injection (Hajishafiha et al., 2011).

This study reports a prospective investigation into whether maternal *H.pylori* seropositivity, detected during the first half of pregnancy, could be associated with the development of the major pregnancy-related pathologies during late second or third trimester, in a general population.

2. Materials and methods

2.1. SUBJECTS and DATA COLLECTION

The study population included all consecutive pregnant women who had routine blood tests at the Laboratory of O.I.R.M. - Sant'Anna Hospital of Turin, between October 2008 and August 2010, before 20 weeks of gestational age. Any multiple pregnancies were excluded. This prospective cohort study was supported by The Italian Ministry of Health (No. RFPS-2007-4-638281), approved by our Hospital Ethics Committee and written informed consent was obtained from each subject enrolled. Blood samples were collected at recruitment and individual patient data included maternal demographic characteristics, previous obstetric and medical history, gestational age at recruitment, pre-pregnancy weight and height for body mass index ($\text{BMI} = \text{Kg/m}^2$) calculation, parity and smoking habit during pregnancy.

Follow-up data were collected from the patients' medical records or obtained by a postnatal interview one month after delivery i.e. gestational age and gestational weight gain at delivery, blood pressure measurements, urinary protein levels, any complications during pregnancy, mode of delivery, neonatal sex and birth weight (neonatal weight was also expressed as centiles, according to birth-weight references for the Italian population) (Bertino et al., 2010).

2.2. DEFINITIONS

Miscarriage included spontaneous miscarriage and/or fetal death before 23 weeks. *Pre-eclampsia* (PE) was defined as the onset of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 300 mg/24h) after 20 weeks of gestational age in previously normotensive women, or a "new-onset proteinuria" in a woman with hypertension before 20 weeks of gestation, a sudden increase in proteinuria if it were already present in early gestation, or the development of HELLP syndrome (ACOG practice bulletin, 2002). PE was considered *severe* when one or more of the following criteria were present: systolic pressure ≥ 160 mmHg or diastolic pressure ≥ 110 mmHg on two occasions at least 6 hrs. apart, or significant proteinuria ($\geq 3+$ on urine dipstick or >5 g in a 24-hour urine), oliguria of <500 mL in 24 hours, cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper-quadrant pain, impaired liver function or thrombocytopenia (ACOG practice bulletin, 2002). Patients with PE were further classified as either having *early-onset* (<34 weeks), or *late-onset* (≥ 34 weeks), disease according to the gestational age when PE was diagnosed. The *HELLP syndrome* was defined by the following criteria: hemolysis (characteristic peripheral blood smear and serum lactate dehydrogenase ≥ 600 U/L), elevated liver enzymes (serum aspartate aminotransferase ≥ 70 U/L) and low platelet count ($<100,000/\mu\text{L}$) (Audibert et al., 1996). *Pregnancy-induced hypertension* (PIH) was diagnosed when hypertension appeared after 20 weeks of gestational age in the

absence of significant proteinuria. *Gestational diabetes mellitus* (GDM) was defined as glucose intolerance with onset or first recognition during pregnancy. The diagnosis of GDM was based on the result of the 100g, 3 hour oral glucose tolerance test (2011). *Obstetric cholestasis* was diagnosed on the basis of the information obtained by clinical examination (generalized pruritus in the absence of any dermatologic condition), laboratory testing that showed a cholestatic pattern (abnormal liver function tests and serum total bile acids exceeding 10 $\mu\text{mol/L}$) that returned to normal values after delivery (Gynaecologists, 2011). *Spontaneous preterm deliveries* included those with spontaneous onset of labour and those with preterm pre-labour rupture of membranes before 37 weeks of gestation.

The term *Appropriate for gestational age* (AGA) refers to neonates with a birth-weight between the 10th and the 90th centile according to birth-weight references for the Italian population (Bertino et al., 2010). SGA (*Small for gestational age*) was defined as newborns with a birth-weight between the 5th and the 9th centile. The classification FGR (*Fetal growth restriction*) was used for any pregnancies with very low birth-weight babies (birth-weight below the 5th centile) and/or SGA newborns with abnormal umbilical arteries Doppler flow velocity waveforms (FVWs) (Todros et al., 1996) and/or abnormal uterine artery Doppler FVWs (resistance index of >0.58) (Steel et al., 1990), when reported. *Large for gestational age* (LGA) neonates was used to define newborns with a birth-weight above the 90th centile, according to birth-weight references for the Italian population (Bertino et al., 2010). Gestational age was calculated from the first day of the last menstrual period and confirmed by ultrasound examination before 20 weeks of gestational age.

2.3. SAMPLES

Maternal blood samples (5 ml) were collected before 20 weeks of gestational age. Venous blood samples were collected in Vacutainer tubes (Becton Dickinson, Plymouth, UK) without anticoagulant and the serum was separated by centrifugation immediately after clotting and stored at -20°C until assayed.

2.4. SEROLOGIC TESTS

Both the presence and titer of antibodies (IgG) against *Helicobacter pylori* were determined in each serum sample using the qualitative/quantitative enzyme-linked immunosorbent assay BEIA *Helicobacter pylori* IgG Quant kit (Technogenetics Srl - Bouty Group, Sesto S.Giovanni, Milan, Italy) and an automatic analyzer, DSX® (DYNEX Technologies, Inc., Chantilly, VA). The concentration of IgG antibodies was calculated by a calibration curve expressed in Arbitrary Units/mL (AU/mL). The samples were considered positive for the presence of anti-*Helicobacter pylori* IgG antibodies when results showed > 11.5 AU/mL, as suggested by the test manufacturer. The sensitivity and specificity of the test were 96.25% and 99.1%, respectively, and the intra- and inter-assay coefficients of variation were lower than 10%, as reported by the test manufacturer.

2.5. STATISTICAL ANALYSIS

All data analyses were performed using the SPSS version 21.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were reported as means and standard deviation (SD). The mean values between the two groups were analysed by the Student's *t*-test, after evaluation of variance homogeneity using the Levene's test. Categorical variables were presented as frequencies (percentages) and the different groups were compared by the chi-square test (χ^2) by means of a 2x2 contingency table; Fisher's exact test was used for small sample sizes.

The odds ratios (OR) and 95% confidence intervals (CI), were calculated by a univariable logistic regression analysis to assess the risk of each pregnancy-related pathology associated with the positive test. Multivariable logistic regression analysis was performed to determine any association between *H.pylori* seropositivity and each of the adverse pregnancy outcomes, adjusted for maternal age, pre-pregnancy BMI, continent origin, smoking habit and the presence of maternal risk factors. All tests were two-tailed and the results were considered significant if they had a *P* value of less than 0.05.

3. RESULTS

A total of 3,113 pregnant women were interviewed during the study period and 293 (9.4%) were excluded. These included 67 twin pregnancies, 25 pregnancies terminated either on psychosocial grounds or for structural and/or chromosomal malformations, 178 were lost at follow-up and 23 had been erroneously recruited twice. After exclusions, the study population was made up of 2,820 women recruited. Table 1 summarizes the main characteristics of our population at recruitment and delivery where serologic tests revealed that 804 (28.5%) women included in this study were seropositive for *H.pylori*.

Gestational diabetes mellitus (GDM) was the most common maternal complication during pregnancy ($n = 160$, 5.7%) in our study population and was the only one with a statistically significantly higher percentage of *H.pylori* positive pregnant women i.e. (41.3%) compared to women without GDM (27.7%) ($P < 0.001$; OR = 1.829, 95% CI: 1.320 - 2.533) (Table 2). When GDM alone without any other maternal pregnancy complication (GDM only group) was considered, the significant difference remained (Table 3). After adjustment for confounding factors the difference remained significant both for all GDM cases ($P = 0.010$) and the GDM only group ($P = 0.009$) (Table 4). A significant association between GDM development and maternal age, pre-pregnancy BMI, continent origin, a family history of diabetes and previous pregnancies complicated by GDM was demonstrated by multivariable logistic regression (Table 4). The comparison between *H.pylori* seronegative and seropositive GDM indicated that seropositive mothers who subsequently developed gestational diabetes (seropositive GDM) were younger (33.2 years) and had a higher pre-pregnancy BMI (26.4) than did seronegative GDM women (35.0 years and pre-pregnancy BMI of 23.9) ($P = 0.013$ and $P = 0.004$, respectively) (Supplementary Table 1).

The percentage of *H.pylori* seropositive women with other maternal complications i.e. spontaneous preterm delivery, pregnancy-induced hypertension, pre-eclampsia, miscarriage or obstetric cholestasis, did not significantly differ from the percentage of *H.pylori* seropositive cases observed in women without these disorders during pregnancy (Tables 2-3). When fetal growth was taken into consideration, the percentage of seropositive women with a SGA newborn was significantly lower (20.3%) than the rest of the study population (28.9%) ($P = 0.033$; OR = 0.626, 95% CI: 0.407 - 0.963) (Table 2). The significant difference remained, when SGA was considered alone without any other pregnancy complication ($P = 0.040$) (Table 3), whilst FGR cases showed no differences for maternal *H.pylori* seropositivity (Table 2-3). Conversely, the significant difference between LGA pregnancies (35.1%) and the other cases (27.9%) ($P = 0.022$) (Table 2) disappeared when LGA was considered without any other complication (Table 3). No association between

H.pylori seropositivity and abnormal fetal growth (SGA, FGR and LGA cases) was observed when data were adjusted for confounding factors (data not shown).

DISCUSSION

Our findings indicate that *H.pylori* maternal seropositivity in the first half of pregnancy is associated with the development of gestational diabetes mellitus (GDM).

The prevalence of *H.pylori* seropositivity in our study population (28.5%) is in line with epidemiologic observations where the authors report a prevalence of 20-30% in middle-aged adults in developed countries (Malaty, 2007).

To the best of our knowledge, this is the first time that a relationship between *H.pylori* seropositivity and GDM development has been demonstrated, while there are several reports that discuss the association between this infection and diabetes mellitus. Some studies have shown a higher prevalence of *H.pylori* infection in people with type 1 (de Luis et al., 1998; El-Eshmawy et al., 2011) and type 2 diabetes (Bener et al., 2007; Devrajani et al., 2010). This relationship seems to be correlated with gender (Hamed et al., 2008), BMI (Bener et al., 2007; Perdichizzi et al., 1996), the duration of diabetes (de Luis et al., 1998), the presence of dyspepsia, cardiovascular autonomic neuropathy (Gulcelik et al., 2005) and glycated hemoglobin (Chen and Blaser, 2012; Hsieh et al., 2013), whilst other studies have indicated neutral or even negative results (Anastasios et al., 2002; Demir et al., 2008; Gillum, 2004). These contradictory findings might well be due to the different study populations. Indeed, negative results were often reported when patients with gastrointestinal symptoms had been investigated. A recent meta-analysis of 41 observational studies has reported a positive association between *H.pylori* infection and type 2 diabetes, whilst no difference in the rate of *H.pylori* infection was demonstrated between type 1 diabetes patients and the non-diabetic control group (Zhou et al., 2013).

The interpretation of the association between diabetes mellitus and *H.pylori* infection is also controversial. On the one hand, some authors report that diabetic patients seem to be more prone to acquire *H.pylori* infection since diabetes mellitus induces impairment of cellular and humoral immunity, enhancing sensitivity to infections (Koh et al., 2012). Others report that diabetes induces a reduction in gastrointestinal motility and acid secretion, thus promoting pathogen colonization and infection rate in the gut (De Block et al., 2006). Perdichizzi *et al.* state that altered glucose metabolism may produce chemical changes in the gastric mucosa that promote *H.pylori* colonization (Perdichizzi et al., 1996). On the other hand, it has been stated that *H.pylori* positivity could be a risk factor for diabetes development, since *H.pylori* infection increases pro-inflammatory cytokine secretion and reactive oxygen species and leads to changes in the structure of the insulin receptor substrate interfering with the interaction with its receptors and inhibiting insulin activity (Aslan et al., 2006; Evans et al., 2002; Marette, 2002). It has also been reported that *H.pylori* infection plays a role in the regulation of leptin and ghrelin (Francois et al., 2011), two hormones involved in energy homeostasis where their interaction affects obesity, insulin sensitivity and glucose homeostasis (Sun et al., 2006; Williams and Mobarhan,

2003). Furthermore, *H.pylori* eradication seems to have beneficial effects on insulin resistance, lipid abnormalities and low-grade inflammation (Gen et al., 2010).

Our findings seem to support the second hypothesis, as, in our study, serum antibodies against *H.pylori* were detected before GDM development and it is likely that *H.pylori* infection occurred before pregnancy. Indeed, *H.pylori* is usually acquired during childhood (Malaty, 2007). Jeon and colleagues also observed the presence of anti-*H.pylori* antibodies before diabetes onset in an elderly population (Jeon et al., 2012). Therefore, it is likely that *H.pylori* positivity predisposes pregnant women for GDM development and the general population for type 2 diabetes mellitus.

We observed that there was a higher percentage of *H.pylori* seropositive women with miscarriages (40.5%) than in the rest of our population (28.3%), without reaching statistical significance. However, we previously reported that if we considered only primigravidae, the difference became significant (Cardaropoli et al., 2013). These findings suggest a relationship between *H.pylori* infection and implantation/placentation failure, possibly due to a cross-reaction between antibodies against *H.pylori* and placental tissue (Franceschi et al., 2012).

Unfortunately, we could not confirm the relationship between *H.pylori* infection and pre-eclampsia (PE) previously reported by us (Cardaropoli et al., 2011; Ponzetto et al., 2006). This is most likely due to the small number of mothers who developed PE (2%), despite the large number of women recruited before 20 weeks of gestation. Furthermore, our PE group was particularly heterogeneous and did not mirror the previous study groups. Indeed, the PE subjects in our previous case-control studies were mainly mothers with a severe/early-onset pre-eclampsia with no other pregnancy-complications (Cardaropoli et al., 2011). In the present investigation there were only 15 (26%) pregnant women with the same characteristics and, in this relatively small subgroup, 7 (46.7%) mothers were *H.pylori* seropositive. However, there may be a second explanation for these conflicting results i.e. *H.pylori* infection might occur during pregnancy shortly before the onset of PE symptoms, therefore there is no seropositivity before 20 weeks. The study carried out by Lanciers and colleagues (1999) support this hypothesis reporting a significantly increased incidence of pregnant subjects with high *H.pylori* IgM (marker for recently acquired infection) than that observed in non pregnant women. Indeed, these authors suggested that pregnancy itself may increase the susceptibility to *H.pylori* infection (Lanciers et al., 1999). We did not demonstrate any relationship between *H.pylori* infection and obstetric cholestasis, spontaneous preterm delivery, pregnancy-related hypertension or fetal growth disorders.

This prospective cohort study faces a pre-eminent problem of modern obstetrics, since GDM increases the risk of complications for both mother and child not only during pregnancy and childbirth but also later in life. In fact, besides the changes in glucose homeostasis, pregnant women with previous GDM are more prone to hypertension,

hyperlipidemia, cardiovascular alterations and mortality. Furthermore, GDM exposes infants to hyperglycaemia whilst *in utero* and it has been reported that this exposure may have a long-term negative impact on the cardiovascular health of the offspring (Marco et al., 2012).

In conclusion, our data demonstrated for the first time that there is an independent association between the presence of antibodies against *H.pylori* antigens in maternal serum and the development of gestational diabetes mellitus.

This is a further confirmation that *H.pylori* infection can have an adverse effect on pregnancy. Therefore, if *H.pylori* infection were to be confirmed as an important risk factor for pregnancy complications, we are of the opinion that pre-pregnancy diagnosis and preventive *H.pylori* eradication would be able to help reduce the incidence of some of these complications. Further studies are ongoing to provide more information on the possible role of screening for *H.pylori* infection in preventing GDM and other serious pregnancy disorders.

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REFERENCES

2011. Committee opinion no. 504: screening and diagnosis of gestational diabetes mellitus. *Obstet Gynecol* 118, 751-753.
- ACOG practice bulletin, 2002. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet. Gynecol.* 99, 159-167.
- Anastasios, R., Goritsas, C., Papamihail, C., Trigidou, R., Garzonis, P., Ferti, A., 2002. *Helicobacter pylori* infection in diabetic patients: prevalence and endoscopic findings. *Eur. J Intern. Med.* 13, 376.
- Aslan, M., Horoz, M., Nazligul, Y., Bolukbas, C., Bolukbas, F.F., Selek, S., Celik, H., Erel, O., 2006. Insulin resistance in *H pylori* infection and its association with oxidative stress. *World J. Gastroenterol.* 12, 6865-6868.
- Audibert, F., Friedman, S.A., Frangieh, A.Y., Sibai, B.M., 1996. Clinical utility of strict diagnostic criteria for the HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome. *Am. J. Obstet Gynecol.* 175, 460-464.
- Banić, M., Franceschi, F., Babić, Z., Gasbarrini, A., 2012. Extragastric manifestations of *Helicobacter pylori* infection. *Helicobacter* 17 Suppl 1, 49-55.
- Bener, A., Micallef, R., Afifi, M., Derbala, M., Al-Mulla, H.M., Usmani, M.A., 2007. Association between type 2 diabetes mellitus and *Helicobacter pylori* infection. *Turk. J. Gastroenterol.* 18, 225-229.
- Bertino, E., Spada, E., Occhi, L., Coscia, A., Giuliani, F., Gagliardi, L., Gilli, G., Bona, G., Fabris, C., De Curtis, M., Milani, S., 2010. Neonatal anthropometric charts: the Italian neonatal study compared with other European studies. *J. Pediatr. Gastroenterol. Nutr.* 51, 353-361.
- Cardaropoli, S., Piazzese, A., Piccoli, E., Rolfo, A., Todros, T., 2013. Is *Helicobacter pylori* infection a risk factor for miscarriage? *Placenta* 34, A37-A38.
- Cardaropoli, S., Rolfo, A., Piazzese, A., Ponzetto, A., Todros, T., 2011. *Helicobacter pylori*'s virulence and infection persistence define pre-eclampsia complicated by fetal growth retardation. *World J. Gastroenterol.* 17, 5156-5165.
- Cardaropoli, S., Rolfo, A., Todros, T., 2014. *Helicobacter pylori* and pregnancy-related disorders. *World J. Gastroenterol.* 20, 654-664.
- Chen, Y., Blaser, M.J., 2012. Association between gastric *Helicobacter pylori* colonization and glycated hemoglobin levels. *J. Infect. Dis.* 205, 1195-1202.
- De Block, C.E., De Leeuw, I.H., Pelckmans, P.A., Van Gaal, L.F., 2006. Current concepts in gastric motility in diabetes mellitus. *Curr. Diabetes Rev.* 2, 113-130.
- de Luis, D.A., de la Calle, H., Roy, G., de Argila, C.M., Valdezate, S., Canton, R., Boixeda, D., 1998. *Helicobacter pylori* infection and insulin-dependent diabetes mellitus. *Diabetes Res. Clin. Pract.* 39, 143-146.
- Demir, M., Gokturk, H.S., Ozturk, N.A., Kulaksizoglu, M., Serin, E., Yilmaz, U., 2008. *Helicobacter pylori* prevalence in diabetes mellitus patients with dyspeptic symptoms and its relationship to glycemic control and late complications. *Dig. Dis. Sci.* 53, 2646-2649.
- Devrajani, B.R., Shah, S.Z., Soomro, A.A., Devrajani, T., 2010. Type 2 diabetes mellitus: A risk factor for *Helicobacter pylori* infection: A hospital based case-control study. *Int. J. Diabetes Dev. Ctries.* 30, 22-26.
- El-Eshmawy, M.M., El-Hawary, A.K., Abdel Gawad, S.S., El-Baiomy, A.A., 2011. *Helicobacter pylori* infection might be responsible for the interconnection between type 1 diabetes and autoimmune thyroiditis. *Diabetol. Metab. Syndr.* 3, 28.
- Evans, J.L., Goldfine, I.D., Maddux, B.A., Grodsky, G.M., 2002. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr. Rev.* 23, 599-622.

- Franceschi, F., Di Simone, N., D'Ippolito, S., Castellani, R., Di Nicuolo, F., Gasbarrini, G., Yamaoka, Y., Todros, T., Scambia, G., Gasbarrini, A., 2012. Antibodies anti-CagA cross-react with trophoblast cells: a risk factor for pre-eclampsia? *Helicobacter* 17, 426-434.
- Francois, F., Roper, J., Joseph, N., Pei, Z., Chhada, A., Shak, J.R., de Perez, A.Z., Perez-Perez, G.I., Blaser, M.J., 2011. The effect of *H. pylori* eradication on meal-associated changes in plasma ghrelin and leptin. *B.M.C. Gastroenterol.* 11, 37.
- Gen, R., Demir, M., Ataseven, H., 2010. Effect of *Helicobacter pylori* eradication on insulin resistance, serum lipids and low-grade inflammation. *South. Med. J.* 103, 190-196.
- Gillum, R.F., 2004. Infection with *Helicobacter pylori*, coronary heart disease, cardiovascular risk factors, and systemic inflammation: the Third National Health and Nutrition Examination Survey. *J. Natl. Med. Assoc.* 96, 1470-1476.
- Gulcelik, N.E., Kaya, E., Demirbas, B., Culha, C., Koc, G., Ozkaya, M., Cakal, E., Serter, R., Aral, Y., 2005. *Helicobacter pylori* prevalence in diabetic patients and its relationship with dyspepsia and autonomic neuropathy. *J. Endocrinol. Invest.* 28, 214-217.
- Gynaecologists, R.C.o.O.a., 2011. Obstetric cholestasis. (Green-top 43). Greentop guideline 43, 1-14.
- Hajishafiha, M., Ghasemi-Rad, M., Memari, A., Naji, S., Mladkova, N., Saeedi, V., 2011. Effect of *Helicobacter pylori* infection on pregnancy rates and early pregnancy loss after intracytoplasmic sperm injection. *Int. J. Womens Health* 3, 329-335.
- Hamed, S.A., Amine, N.F., Galal, G.M., Helal, S.R., Tag El-Din, L.M., Shawky, O.A., Ahmed, E.A., Abdel Rahman, M.S., 2008. Vascular risks and complications in diabetes mellitus: the role of *helicobacter pylori* infection. *J. Stroke Cerebrovasc. Dis.* 17, 86-94.
- Hsieh, M.C., Wang, S.S., Hsieh, Y.T., Kuo, F.C., Soon, M.S., Wu, D.C., 2013. *Helicobacter pylori* infection associated with high HbA1c and type 2 diabetes. *Eur. J. Clin. Invest.* 43, 949-956.
- Jeon, C.Y., Haan, M.N., Cheng, C., Clayton, E.R., Mayeda, E.R., Miller, J.W., Aiello, A.E., 2012. *Helicobacter pylori* infection is associated with an increased rate of diabetes. *Diabetes Care* 35, 520-525.
- Koh, G.C., Peacock, S.J., van der Poll, T., Wiersinga, W.J., 2012. The impact of diabetes on the pathogenesis of sepsis. *Eur. J. Clin. Microbiol. Infect. Dis.* 31, 379-388.
- Lanciers, S., Despinasse, B., Mehta, D.I., Blecker, U., 1999. Increased susceptibility to *Helicobacter pylori* infection in pregnancy. *Infect. Dis. Obstet. Gynecol.* 7, 195-198.
- Malaty, H.M., 2007. Epidemiology of *Helicobacter pylori* infection. *Best Pract. Res. Clin. Gastroenterol.* 21, 205-214.
- Marco, L.J., McCloskey, K., Vuillermin, P.J., Burgner, D., Said, J., Ponsonby, A.L., 2012. Cardiovascular disease risk in the offspring of diabetic women: the impact of the intrauterine environment. *Exp. Diabetes Res.* 2012, 565160.
- Marette, A., 2002. Mediators of cytokine-induced insulin resistance in obesity and other inflammatory settings. *Curr. Opin. Clin. Nutr. Metab. Care* 5, 377-383.
- Perdichizzi, G., Bottari, M., Pallio, S., Fera, M.T., Carbone, M., Barresi, G., 1996. Gastric infection by *Helicobacter pylori* and antral gastritis in hyperglycemic obese and in diabetic subjects. *New Microbiol.* 19, 149-154.
- Ponzetto, A., Cardaropoli, S., Piccoli, E., Rolfo, A., Gennero, L., Kanduc, D., Todros, T., 2006. Pre-eclampsia is associated with *Helicobacter pylori* seropositivity in Italy. *J. Hypertens.* 24, 2445-2449.
- Steel, S.A., Pearce, J.M., McParland, P., Chamberlain, G.V., 1990. Early Doppler ultrasound screening in prediction of hypertensive disorders of pregnancy. *Lancet* 335, 1548-1551.
- Sun, Y., Asnicar, M., Saha, P.K., Chan, L., Smith, R.G., 2006. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell. Metab.* 3, 379-386.

Todros, T., Ronco, G., Fianchino, O., Rosso, S., Gabrielli, S., Valsecchi, L., Spagnolo, D., Acanfora, L., Biolcati, M., Segnan, N., Pili, G., 1996. Accuracy of the umbilical arteries Doppler flow velocity waveforms in detecting adverse perinatal outcomes in a high-risk population. *Acta Obstet. Gynecol. Scand.* 75, 113-119.

Williams, J., Mobarhan, S., 2003. A critical interaction: leptin and ghrelin. *Nutr. Rev.* 61, 391-393.

Zhou, X., Zhang, C., Wu, J., Zhang, G., 2013. Association between *Helicobacter pylori* infection and diabetes mellitus: a meta-analysis of observational studies. *Diabetes Res. Clin. Pract.* 99, 200-208.

HIGHLIGHTS

- *H.pylori* seropositivity before 20 wks is associated with gestational diabetes.
- Maternal *H.pylori* seropositivity is independently associated with GDM development.
- *H.pylori* eradication should prevent diabetes mellitus.

Table 1. The characteristics of all recruited cases.

Characteristic	Mean (SD)
<i>At recruitment</i>	
Maternal age (years)	32.2 (4.5)
Gestational age (weeks)	13.3 (2.4)
<i>At delivery</i>	
Gestational weight gain (Kg)	13.0 (4.6)
Rate of weight gain (Kg/week)	0.34 (0.12)
Gestational age (weeks)	38.6 (3.2)
Neonatal birth weight (g)	3,239 (486)
Neonatal birth weight centile	47.6 (27.8)
<i>N (%)</i>	
<i>Continent origin</i>	
European	2,693 (95.5)
Non european	127 (4.5)
<i>Pre-pregnancy Body Mass Index</i>	
Underweight (BMI < 18,5)	230 (8.2)
Normal (18,5 ≤ BMI < 25)	2,009 (71.3)
Overweight (25 ≤ BMI < 30)	435 (15.4)
Obese (BMI ≥ 30)	145 (5.1)
<i>Nulliparous</i>	1,689 (59.9)
<i>Smoking mothers</i>	321 (11.4)
<i>Pre-pregnancy pathologies</i>	
Chronic hypertension	51 (1.8)
Diabetes mellitus	16 (0.6)
Nephropathy	20 (0.7)
Thrombophilia	62 (2.2)
Autoimmune diseases	66 (2.3)
<i>H.pylori seropositivity</i>	804 (28.5)

Table 2. Univariable logistic regression analysis on the association between the presence of *H.pylori* antibodies in maternal serum and major pregnancy-related complications.

Outcome	Total N (%)	Seropositive N (%)	<i>P</i> value	OR (95% CI)
All GDM cases	160 (5.7)	66 (41.3)	<0.001	1.829 (1.320 – 2.533)
Pregnancies without GDM	2,660 (94.3)	738 (27.7)		
All spontaneous preterm deliveries	100 (3.5)	36 (36.0)	0.093	1.430 (0.942 – 2.169)
No spontaneous preterm delivery	2,720 (96.5)	768 (28.2)		
All PIH cases	140 (5.0)	35 (25.0)	0.346	0.828 (0.560 – 1.225)
Pregnancies without PIH	2,680 (95.0)	769 (28.7)		
All PE cases	57 (2.0)	16 (28.1)	0.941	0.978 (0.546 – 1.753)
Pregnancies without PE	2,763 (98.0)	788 (28.5)		
All miscarriage cases	37 (1.3)	15 (40.5)	0.107	1.723 (0.889 – 3.339)
Pregnancies without miscarriage	2,783 (98.7)	789 (28.3)		
All Cholestasis cases	34 (1.2)	8 (23.5)	0.519	0.769 (0.347 – 1.706)
Pregnancies without Cholestasis	2,786 (98.8)	796 (28.6)		
All SGA cases	133 (4.7)	27 (20.3)	0.033	0.626 (0.407 – 0.963)
Pregnancies with no SGA neonates	2,687 (95.3)	777 (28.9)		
All FGR cases	131 (4.6)	45 (34.4)	0.131	1.331 (0.919 – 1.927)
Pregnancies with no FGR neonates	2,689 (95.4)	759 (28.2)		
All LGA cases	231 (8.2)	81 (35.1)	0.022	1.394 (1.050 – 1.851)
Pregnancies with no LGA neonates	2,589 (91.8)	723 (27.9)		

Table 3. Univariable logistic regression analysis on the association between the presence of *H.pylori* antibodies in maternal serum and major pregnancy-related disorders without any other maternal complications.

Outcome	Total	Seropositive	<i>P</i> value	OR (95% CI)
	N (%)	N (%)		
GDM	136 (4.8)	58 (42.6)	<0.001	1.932 (1.361 – 2.742)
Spontaneous preterm	75 (2.7)	23 (30.7)	0.675	1.112 (0.676 – 1.830)
PIH	124 (4.4)	30 (24.2)	0.277	0.793 (0.521 – 1.205)
PE	48 (1.7)	13 (27.1)	0.825	0.930 (0.490 – 1.768)
Cholestasis	27 (1.0)	4 (14.8)	0.124	0.433 (0.149 – 1.257)
SGA	107 (3.8)	21 (19.6)	0.040	0.602 (0.371 – 0.977)
FGR	103 (3.7)	34 (33.0)	0.304	1.246 (0.819 – 1.895)
LGA	182 (6.5)	62 (34.1)	0.087	1.320 (0.961 – 1.815)

Table 4. Multivariable logistic regression analysis on the association between the presence of *H.pylori* antibodies in maternal serum and major pregnancy-related complications.

Independent variable	GDM ^a		GDM only ^b	
	p value	OR (95% CI)	p value	OR (95% CI)
<i>H.pylori</i> seropositivity	0.010	1.599 (1.121-2.280)	0.009	1.655 (1.133-2.416)
Maternal age	<0.001	1.138 (1.091-1.186)	<0.001	1.143 (1.093-1.195)
Body mass index	<0.001	1.113 (1.074-1.152)	<0.001	1.089 (1.047-1.132)
Non European	0.035	2.055 (1.053-4.010)	0.008	2.486 (1.269-4.872)
Cigarette smoking	0.479	1.206 (0.718-2.026)	0.696	1.121 (0.633-1.984)
Family history for diabetes	0.028	1.572 (1.051-2.354)	0.149	1.387 (0.889-2.163)
Family history for hypertension	0.263	0.820 (0.580-1.161)	0.291	0.818 (0.564-1.187)
Chronic hypertension	0.828	0.890 (0.311-2.547)	0.973	0.981 (0.315-3.053)
Autoimmune disease	0.235	1.723 (0.702-4.231)	0.609	1.319 (0.456-3.814)
Nephropathy	0.998	0.000 (0.000-∞)	0.998	0.000 (0.000-∞)
<i>Parity</i>				
Nulliparous	<0.001	1	<0.001	1
Parous w/o previous GDM	0.012	1.601 (1.107-2.316)	0.099	1.391 (0.940-2.058)
Parous with previous GDM	<0.001	13.250 (6.031-29.109)	<0.001	11.641 (5.248-25.821)

^a with or without any other maternal complication; ^b without any other maternal complication

Supplementary Table 1. Comparison between *H.pylori* seronegative and seropositive GDM.

	GDM	
	Seronegative	Seropositive
	(n.94)	(n.66)
	<i>Mean (SD)</i>	<i>Mean (SD)</i>
Maternal age (years)	35.0 ^a (3.9)	33.2 (4.9)
Gestational age at recruitment (weeks)	13.0 (2.4)	13.3 (3.3)
Pre-pregnancy BMI (Kg/m ²)	23.9 ^b (4.7)	26.4 (6.3)
Gestational age at delivery (weeks)	38.5 (1.4)	38.6 (1.5)
Gestational weight gain (Kg)	10.5 (5.1)	10.8 (5.0)
Neonatal birth weight (g)	3,219 (507)	3,327 (481)
Neonatal birth weight centile	49.1 (28.3)	56.5 (29.2)
	<i>N (%)</i>	<i>N (%)</i>
<i>Continent origin</i>		
European	90 (95.7)	58 (87.9)
Non european	4 (4.3)	8 (12.1)
Smoking mothers	14 (14.9)	5 (7.6)
<i>Parity</i>		
Nulliparous	51 (54.3)	37 (56.1)
Parous w/o previous GDM	34 (36.2)	21 (31.8)
Parous with previous GDM	9 (9.6)	8 (12.1)
Chronic hypertension	1 (1.1)	4 (6.1)
Autoimmune disease	7 (7.4)	8 (12.1)
<i>Therapy</i>		
Diet	85 (90.4)	60 (90.9)
Insulin	9 (9.6)	6 (9.1)
<i>Other complications</i>		
Without other complications	78 (83.0)	58 (87.9)

With other complications	16 (17.0)	8 (12.1)
<i>with Pregnancy-induced hypertension</i>	9 (9.6)	4 (6.1)
<i>with Pre-eclampsia</i>	6 (6.4)	2 (3.0)
<i>with Obstetric cholestasis</i>	1 (1.1)	2 (3.0)
<i>Neonatal birth weight</i>		
AGA	78 (83.0)	52 (78.8)
SGA	4 (4.3)	2 (3.0)
FGR	2 (2.1)	2 (3.0)
LGA	10 (10.6)	10 (15.2)

^a*P* value from Student's t-test = 0.013; ^b*P* value from *Chi*-square test = 0.004