

High Levels of IgA Antibodies to *Helicobacter Pylori* among Omani Women during Pregnancy and after Delivery

Mohammed Said Al-Balushi^{1#*}, Elias Anthony Said^{1#}, Sidgi Syed Hasson¹, Juma Zaid Al-Busaidi¹, Iman Al-Reesi¹, Mohammed Idris¹, Wadha Al-Ghafri², Moza Al-Kalbani², Ali Abdullah Al-Jabri¹

¹Department of Microbiology and Immunology, ²Department of Obstetrics and Gynaecology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman

ABSTRACT

Background: *Helicobacter pylori* (*H. pylori*), is a common infection in pregnant women accompanied by variations in the levels of the IgM, IgA and IgG antibody isotypes. The variations of anti-*H. pylori* antibodies during and after pregnancy, and the extent of protection they provide to the mother and the fetus are not completely understood. **Objectives:** To investigate the changes of the anti-*H. pylori* IgM, IgA and IgG levels in healthy Omani pregnant women during pregnancy and 3 months after delivery. **Methods:** Serum samples obtained from 70 Omani healthy pregnant women, with no history of autoimmune diseases, were tested for anti-*H. pylori* IgM, IgA and IgG in the first trimester of pregnancy and 3 months after delivery. In parallel and as a control group, sera obtained from a group of 70 healthy non-pregnant Omani women were tested. The levels of anti-*H. pylori* IgM, IgA and IgG were measured using standard Enzyme Linked Immunosorbent Assays (ELISAs). **Results:** Anti-*H. pylori* IgA levels were found to be significantly higher during pregnancy ($p=0.046$) and after delivery ($p=0.02$) when compared to the control group. Moreover, a significant increase in the levels of anti-*H. pylori* IgM, IgA and IgG was detected after delivery ($p=0.002$) when compared to the levels during pregnancy. **Conclusion:** Pregnancy is associated with an increase in the levels of anti-*H. pylori* IgA antibodies. In addition, anti-*H. pylori* IgM, IgG and IgA antibody levels increase after delivery.

Al-Balushi M., et al. *Iran J Immunol.* 2016; 13(2):114-123.

Keywords: Antibodies, *Helicobacter pylori*, IgA, Infection, Pregnancy, Women

*Corresponding author: Dr. Mohammed Al-Balushi, Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman, Tel: (+) 96 8 24141179, Fax: (+) 968 24413300. e-mail: albuloshi@hotmail.com

#These authors contributed equally to this work

INTRODUCTION

Pregnancy is associated with a large number of immunological changes and adaptations which prompts the increased susceptibility to various infectious agents. During pregnancy, the maternal immune system provide protection for the growing placenta and fetus (1).

Alterations in the various isotypes of antibodies (IgM, IgA and IgG) occurs during the pregnancy period (2). IgM is the first antibody isotype to respond when a foreign antigen is detected in the body. Due to its large size IgM cannot pass through the placenta from the mother to the fetus and it is produced by the fetus in response to infection at the age of 5 months of pregnancy (3) Thus, any IgM presence in the circulation of a new-born indicates an infection has occurred during pregnancy (4).

IgA provides mucosal defences against any infection and therefore its levels would be expected to increase in pregnant woman with an infection (5). Similarly, the concentrations of IgG would be expected to be greater in infected pregnant women (6). Maternal IgG is the only transplacental antibody that provides protection to the fetus as the humoral immunity of the fetus is functionally immature (7).

Certain infections including *H. pylori* may be acquired during pregnancy and may have severe complications, clinical course and higher fatality rates (8-10). *H. pylori* is a gram-negative bacterium that infects over half of the world population (11). *H. pylori* infection is known to be associated with gastric cancer, chronic gastritis, peptic ulcers, duodenal ulcers, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (12-13). *H. pylori* is an important cause of gastritis in children. It increases gastroesophageal reflux disease and the risk of developing gastric cancer. The risk of infection with *H. pylori* in infants is about 3.4 times higher if the mother is also infected (14).

In this study we aim to investigate the levels of anti-*H. pylori* specific to IgM, IgA and IgG antibody isotypes in healthy pregnant women and 3 months after delivery.

MATERIALS AND METHODS

Study Population. A total of 140 Omani women were enrolled in this longitudinal cohort study. Of all women, 70 were healthy pregnant women (average age = 26 ± 3 years) who attended the antenatal care outpatient clinic of the Department of Obstetrics and Gynaecology at the Armed Forces Hospital (AFH) in Muscat, Oman; $\approx 80\%$ of the pregnant women had been pregnant before the current pregnancy. The other 70 were healthy women (average age = 24 ± 2 years), who have never been pregnant and entered the study in parallel as a control group. Individuals enrolled in this study did not have any connective tissue disease or other autoimmune diseases, previous history of thrombo-embolism, recurrent abortions and treatments affecting the immune response, such as corticosteroids, immunosuppressive drugs or immune-modulators. All the pregnant women were followed up over the whole period of pregnancy and after delivery. All the included pregnant women had uncomplicated and normal pregnancy outcome.

Informed consent was obtained from all participants and ethical clearance was obtained from the Ethics Committee of the Sultan Qaboos University, Muscat, Oman (MREC #

654). To maintain confidentiality, each participant was assigned with a unique identification number.

Blood Collection and Measurement of Antibody Levels. Blood samples of 8 ml were collected from all the 70 pregnant women during the first trimester of pregnancy and after three months of delivery. In parallel, 8 ml blood samples were also collected from a total of 70 healthy non-pregnant women used as a control group. All samples were collected in serum-Plus Blood Collection Tubes (BD Vacutainer, USA).

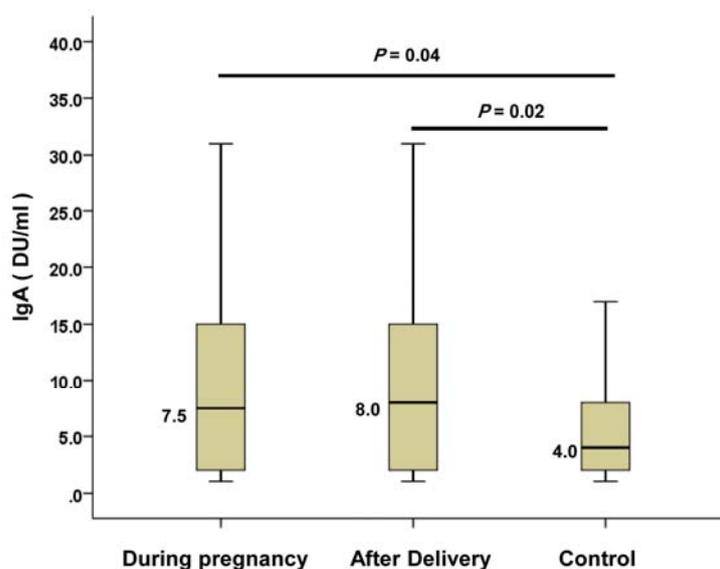
Sera were separated after centrifugation at $500 \times g$ in a cooling centrifuge. All sera were tested for the levels of *H. pylori* specific to IgG and IgA antibodies using standard ELISA and IgM using EIA (DRG International Inc., USA) following manufacturer's instructions. IgG and IgA levels were expressed in DU/ml (DU = DRG Units) and IgM in EIA units. *H. pylori* IgG and IgA were considered as positive when their cut-off values ≥ 20 DU/ml, and it was ≥ 1 EIA Index for IgM. The optical density of the different ELISA samples was read using Absorbance Reader (Biotek ELX800, USA).

Statistical Analysis. Mann-Whitney U test was used to assess the significance of the changes in the levels of antibodies obtained from the pregnant women during their pregnancy and after delivery when compared to the control group. Furthermore, Wilcoxon signed-rank test was performed to assess the significance of the increase in the levels of antibodies during pregnancy and after delivery in the same group. Spearman Rank correlation test was used to evaluate the significance of the correlations between the levels of the antibody isotypes during pregnancy and after delivery. The differences in antibody levels and the correlations were considered significant when the p value was < 0.05 . Statistical analyses were performed using Statistical Package for Social Sciences (SPSS, v21.0) and GraphPad Prism v6.

RESULTS

Pregnancy is Associated with an Elevation in the Levels of Anti-*H. pylori* IgA. We compared the levels of anti-*H. pylori* IgM, IgA and IgG in non-pregnant women with the levels of these antibodies during pregnancy and after delivery.

Figure 1: Difference in the IgA levels specific to *H. pylori* during pregnancy and after delivery compared with the control. Blood samples were collected from 70 pregnant women during the first trimester of pregnancy and after three months of delivery. Sera were tested for the levels of *H. pylori* specific IgA antibodies using standard ELISA. Data are expressed as mean \pm SD. Statistical analysis Mann-Whitney U test. The horizontal bars indicate the mean value of each sample group.



IgA levels during pregnancy and after delivery were significantly higher than in the control group, ($p=0.046$ and 0.02 , respectively, Figure 1).

The IgA levels during pregnancy (median=7.5 DU/ml) and after delivery (median = 8.0 DU/ml) showed a significant increase compared to the control group (mean = 4.0 DU/ml). Further, no significant difference was seen in the levels of IgG and IgM antibodies when comparing their levels in the different groups (data not shown).

Significant Increase in Anti-*H. pylori* IgM, IgA and IgG Levels after Delivery. We explored the changes in the levels of each of the three isotypes of the anti-*H. pylori* antibodies by comparing their levels in each patient (paired test) during pregnancy and after delivery. The average IgM levels during pregnancy (0.88 DU/ml) was lower than that after delivery (0.9 DU/ml; $p=0.031$, Figure 2A and Table 1).

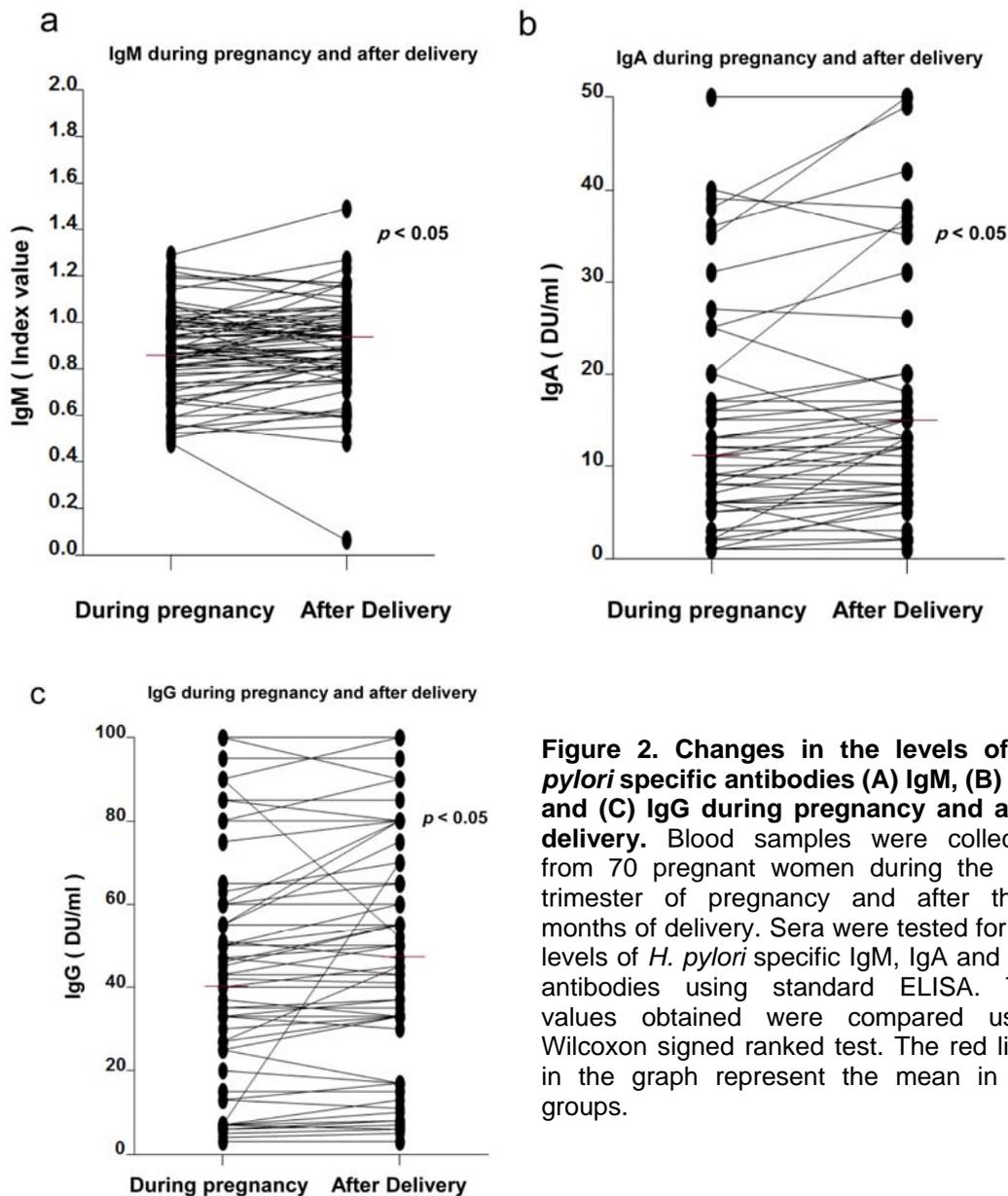


Figure 2. Changes in the levels of *H. pylori* specific antibodies (A) IgM, (B) IgA and (C) IgG during pregnancy and after delivery. Blood samples were collected from 70 pregnant women during the first trimester of pregnancy and after three months of delivery. Sera were tested for the levels of *H. pylori* specific IgM, IgA and IgG antibodies using standard ELISA. The values obtained were compared using Wilcoxon signed ranked test. The red lines in the graph represent the mean in the groups.

Table 1. The mean values of anti-*H. pylori* IgM, IgG and IgA during pregnancy and after delivery. The mean values \pm S.D. are presented. The *p* values for the comparisons between the levels of the antibodies during pregnancy and after delivery are represented in line 3, and those of the correlation between these levels are represented in line 4.

	IgM	IgG	IgA
During pregnancy	0.88 \pm 0.19	41.6 \pm 27.9	11.04 \pm 11.2
After delivery	0.9 \pm 0.21	44.66 \pm 28.2	12.4 \pm 12.6
P value for the increase	0.031	0.002	0.001
P value for the correlation	0.001	0.001	0.001

Comparable results were found in the levels of IgA and IgG, respectively. The average IgA levels after delivery (12.43 DU/ml) was higher compared to the average during pregnancy (11.04 DU/ml; $p=0.002$, Figure 2B and Table 1). Similarly, there was an increase in IgG levels with a mean of 44.66 DU/ml after delivery compared to 41.6 DU/ml during pregnancy ($p=0.001$, Figure 2C and Table 1). When we considered the values that are above the cut-off point only, IgG levels after delivery (42.06 DU/ml) were found to be higher when compared to their levels during pregnancy (39.64 DU/ml; $p=0.001$; Supplementary Figure 1).

The Relationship between Anti-*H. pylori* IgM, IgA and IgG during Pregnancy and after Delivery. We investigated the correlations between anti-*H. pylori* IgM, IgA and IgG levels during pregnancy and after delivery. A significant correlation was observed between IgM levels during pregnancy and their levels after delivery ($\rho = 0.811$; $p = 0.001$ Figure 3A and Table 1). A similar correlation was observed for IgA levels during pregnancy and after delivery ($\rho = 0.939$; $p=0.001$; Figure 3B and Table 1), and for IgG levels during pregnancy and after delivery ($\rho = 0.933$; $p=0.001$; Figure 3C and Table 1). When only IgM, IgA and IgG values that were above the cut-off point were considered, the correlations remained significant for IgM ($\rho = 0.532$; $p = 0.001$; supplementary Figure 2A), IgA ($\rho = 0.845$; $p=0.001$; supplementary Figure 2B) and IgG ($\rho = 0.928$; $p=0.001$; supplementary Figure 2C).

Moreover, we investigated the existence of correlations between the levels of the different *H. pylori*-specific antibody isotypes during pregnancy and after delivery. There was a significant correlation between IgA and IgG levels during pregnancy ($\rho = 0.591$; $p=0.001$, Figure 3D and Table 2), as well as IgA and IgG levels after delivery ($\rho = 0.556$; $p=0.001$ Figure 3G and Table 2). There was a significant correlation between levels of IgA during pregnancy and IgG levels after delivery ($\rho = 0.578$; $p=0.001$; Figure 3E and Table 2), and between IgG levels during pregnancy and IgA levels after delivery, ($\rho = 0.560$; $p=0.001$; Figure 3F and Table 2). The IgA and IgG levels after delivery also showed a significant correlation ($\rho = 0.556$; $p=0.001$; Figure 3G and Table 2). However, there was no correlation between the IgM levels and both IgA and IgG levels. When we considered the values that were above the cut-off point, a significant

correlation was found between IgA and IgG levels during pregnancy ($\rho = 0.408$; $p=0.001$; supplementary Figure 2D) and IgA during pregnancy and IgG after delivery, ($\rho = 0.378$; $p=0.001$; supplementary Figure 2E). Likewise, levels of IgA after delivery and IgG during pregnancy were significant ($\rho=0.359$; $p=0.002$; supplementary Figure 2F). The correlation between the levels of IgA and IgG after delivery were also significant ($\rho = 0.396$; $p=0.001$; supplementary Figure 2G).

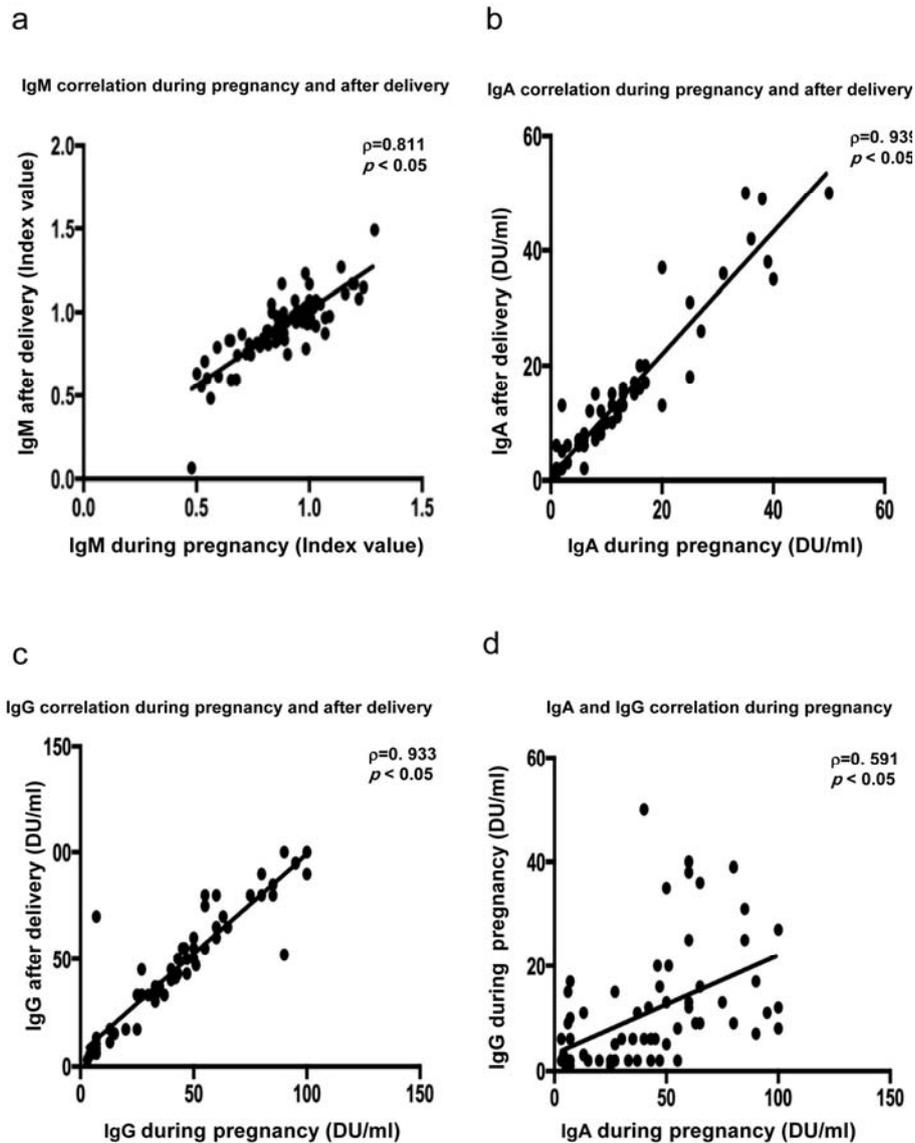


Figure 3. Correlation between Immunoglobulin during pregnancy and after delivery. Blood samples were collected from 70 pregnant women during the first trimester of pregnancy and after three months of delivery. Sera were tested for the levels of *H. pylori* specific IgM, IgA and IgG antibodies using standard ELISA (A) IgM level during pregnancy correlated with IgM level after delivery (B) IgA level during pregnancy correlated with IgA level after delivery (C) IgG level during pregnancy correlated with IgG level after delivery (D) IgA level during pregnancy correlated with IgG level during pregnancy (E) IgG level after delivery correlated with IgA level during pregnancy (F) IgA level after delivery correlated with IgG level during pregnancy (G) IgG level after delivery correlated with IgA level after delivery.

Table 2. The correlation between the levels of anti-*H. pylori* IgG and IgA during pregnancy and after delivery. The p values for the correlations are presented.

	IgA Durign Pregnancy	IgA after Delivery
IgG durign pregnancy	0.001	0.001
IgG after delivery	0.002	0.001

DISCUSSION

The present study showed a significant increase in the levels of IgA against *H. pylori* both during pregnancy and after delivery compared to the control group. This increase in IgA levels may potentially provide an immune protection for the mother in early postpartum stages. Moreover, the high levels of IgA in the maternal serum provide protection against *H. pylori* not only for the mother but also to the neonate (23). During breastfeeding, the maternal IgAs are transferred to the new born and these antibodies may play an important role in the prevention of *H. pylori* infection (24,25). Although another study showed that passively acquired maternal antibodies in infants with high risk of *H. pylori* infection had no protective role. It was suggested that IgA antibodies present in the breast milk give passive protection against early human *H. pylori* colonization (23). However, *H. pylori* prevalence was higher in breastfed babies compared to those who were never breastfed. It was concluded that ingestion of maternal milk may not protect against *H. pylori* infections (11). In fact, the study that showed the role of breastfeeding in the transmission of *H. pylori* did not assess the levels of anti-*H. pylori* antibodies in the maternal milk, therefore it is not clear whether this higher transmission was associated with the absence or low levels of anti-*H. pylori* antibodies in the maternal milk. The presence of an inversed correlation between the levels of anti-*H. pylori* antibodies in the maternal milk and the transmission of *H. pylori* to the child requires further investigation.

IgG levels were similar in the control group and in pregnant women and after delivery, which is consistent with the results reported by Lanciers *et al.* (20). IgG and IgM levels against *H. pylori* were found to be higher after delivery when compared to their levels during pregnancy, indicating the possible activation of new anti-*H. pylori* B lymphocyte responses upon delivery. This activation might be due to the fact that pregnancy is associated with a Th2 cytokine environment (31). An earlier study has found that there is a significant rise in the rate of pregnant women with high levels of *H. pylori* IgM, which is a marker for newly acquired infection, compared to non-pregnant women (15). An increase in the susceptibility to *H. pylori* infection has been associated with pregnancy itself which could be owing to the fact that during pregnancy there are immunological adaptations that ensure maternal tolerance towards the semi-allogeneic fetus (1,16). We did not find any increase in IgM levels when comparing pregnant women to the control group, which may be explained by the absence of new infections with *H. pylori*. Our results suggest that the higher levels of anti-*H. pylori* IgG and IgA

after delivery may provide further protection to both the mother and the new born. It is possible therefore to hypothesise that interventions aiming to boost the production of anti-*H. pylori* antibodies in the mother may provide protection through breastfeeding to the neonates who have an immature immune system. Earlier studies have suggested that maternal IgG along with IgA antibodies in human milk may protect against *H. pylori* infection thus the onset of infection is delayed (26,27). The titre of anti-*H. pylori* IgG antibodies in infants who were positive at the time of delivery, turned negative by the age of 3 months, indicating that these IgG antibodies were from the mother (28). It is well known that anti-*H. pylori* IgG antibodies are transferred through placenta from mothers to fetuses (4). Women having blood group B⁺ have been shown to be more efficient in transferring anti-*H. pylori* IgG to their neonates (29).

This study shows a positive correlation between the levels of anti *H. pylori* IgA during pregnancy and after delivery, which was also observed for both IgM and IgG. This indicates that the magnitude of the B lymphocyte response against *H. pylori* during pregnancy influences its magnitude after delivery. This might be affected by the potency of the B cells producing the anti-*H. pylori* antibodies or the amount of *H. Pylori* antigens to which these B cells are exposed. The levels of IgA and IgG directed against *H. pylori* found to be correlated during pregnancy and after delivery indicating that a similarity in the source and the levels of activation led to the production of these isotypes. This might be due to the fact that the production of both IgA and IgG can be influenced by the Th2 environment established during pregnancy (32).

Specific anti-*H. pylori* IgG antibodies are transplacentally transferred from mother to the fetus and a strong association between maternal and cord specific IgG levels was reported (30). The level of these passively acquired antibodies decreases over the first three to four months of life. It is also suggested that maternal IgG may protect against infections with *H. pylori*, which is supported by findings in murine models (31). Other studies found no confirmation of a protective role for passively acquired maternal antibodies in infants with high risk of *H. pylori* infection (4).

In summary, this study clearly shows that there is a significant increase of anti-*H. pylori* IgA levels during pregnancy and of IgM, IgA and IgG after delivery. Our results suggest that these antibodies may potentially provide protection to the new born against this bacterial infection, however the protective role of these antibodies is controversial as mentioned above (4,31), and more studies are needed to clarify the exact role of these antibodies. To the best of our knowledge, no prior studies reported an elevation in the levels of anti- *H. pylori* IgA in the maternal serum during the first trimester of pregnancy and 3 months after delivery. Our results can be further confirmed using a larger sample size.

ACKNOWLEDGEMENTS

The authors thank the staff in the Department of Microbiology and Immunology at Sultan Qaboos University for technical help. This study was supported by Sultan Qaboos University. Research Grant No. IG/MED/MIR/13/02.

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