Fluctuation in the Levels of Immunoglobulin M and Immunoglobulin G Antibodies for Cardiolipin and β₂-Glycoprotein among Healthy Pregnant Women

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التقلبات في مستويات الأجسام المضادة للكارديوليبين وبيتا2– جليكوبروتين عند النساء الحوامل

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abstract: *Objectives:* Antiphospholipid antibodies fluctuate during a healthy normal pregnancy. This study aimed to investigate the levels of both immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies for cardiolipin and β₂-glycoprotein (β₂GP) among healthy pregnant women. *Methods:* This study was conducted between May 2010 and December 2012. A total of 75 healthy Omani pregnant women with no history of autoimmune disease were investigated during their pregnancy and 90 days after delivery at the Armed Forces Hospital in Muscat, Oman. A control group of 75 healthy Omani non-pregnant women were also investigated as a comparison. Levels of IgM and IgG antibodies for both anti-cardiolipin antibodies (ACAs) and β₂GP were measured using a standard enzyme-linked immunosorbent assay. *Results:* The ACA IgM levels were significantly higher in the control group compared to the pregnant women ($P < 0.001$). No significant differences were observed in the ACA IgM levels between the control group and the pregnant women after delivery. In contrast, ACA IgG levels were significantly higher during pregnancy and after delivery compared with those of the healthy control group ($P = 0.007$ and 0.002, respectively). The levels of β₂GP IgG were significantly higher during pregnancy than after delivery and in the control group ($P = 0.001$ and <0.001, respectively). *Conclusion:* In this study, ACA IgG levels increased during healthy pregnancies and after normal deliveries whereas β₂GP IgG levels increased transiently during the pregnancies. Both phenomena were found to be significantly associated with a transient decline in the levels of IgM specific for these antigens. Therefore, the levels of these antibodies may be regulated during a healthy pregnancy.

Keywords: Anticardiolipin Antibodies; beta 2-Glycoprotein I; Pregnancy; Women; Oman

الملخص: الهدف: مستوى الأجسام المضادة للفوسفوليبيد يتقلب أثناء فترة الحمل الطبيعي. هدفت هذه الدراسة للتحقق من مستويات الأجسام المضادة IgM والأجسام المضادة IgG للكارديوليبين (ACA) و دهون البيتا–2 جليكوبروتين (β₂GP) بين النساء العمانيات الحوامل بحمل صحي سليم. الطريقة: أجريت هذه الدراسة بين أيار/مايو 2010، وكانون الأول/ديسمبر، 2012. وشملت الدراسة مجموعة أولى مكونة من 75 إمرأة عمانية حامل بحمل طبيعي صحي غير مصابات بأمراض المناعة الذاتية وتم تحليل العينات خلال فترة الحمل و 90 يوما بعد الولادة. كما شملت هذه الدراسة 75 من النساء غير الحوامل كمجموعة مراقبة على سبيل المقارنة. تم قياس مستويات الأجسام المضادة الأجسام المضادة للكارديوليبين و بيتا 2–جليكوبروتينباستخدام طريقة الإليزا(ELISA). النتائج: كانت مستويات الأجسام المضادة IgM للكارديوليبين أعلى بكثير في مجموعة المراقبة بالمقارنة مع مستويات IgM في النساء الحوامل (0.001> P). وفي المقابل كانت مستويات IgG للكارديوليبين أعلى بكثير أثناء فترة الحمل وبعد الولادة بالمقارنة مع مجموعة المقارنة (0.007 = P و 0.002 = P، على التوالي).أما الاجسام المضادة IgG لدهون البيتا–2 جليكوبروتين فكانت المستويات أعلى بكثير خلال الحمل من بعد الولادة، وفي مجموعة المقارنة أيضا (0.001 = P و 0.01>، على التوالي). الخلاصة: في هذه الدراسة، وجدت زيادة في مستويات IgG للكارديوليبين أثناء الحمل الطبيعي وبعد الولادات الطبيعية بينما زادت مستويات IgG لدهون البيتا–2 جليكوبروتين زيادة عابرة أثناء فترة الحمل. وتم العثور على كل هذه الظواهر مرتبطة إلى حد كبير مع انخفاض عابر في مستويات محددة لهذه المضادات IgM مما قد ينظم مستويات هذه الأجسام المضادة أثناء فترة الحمل الطبيعي.

مفتاح الكلمات: الأجسام المضادة الكارديوليبين؛ بيتا 2–جليكوبروتين؛ حمل؛ نساء؛ عمان.

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ADVANCES IN KNOWLEDGE

- *Similarities in the fluctuation patterns of cardiolipin and β_2-glycoprotein (β_2GP) autoantibodies were noted among a group of healthy pregnant women.*
- *Levels of both anti-cardiolipin antibodies (ACAs) and β_2GP immunoglobulin G (IgG) were found to increase significantly during healthy normal pregnancies. However, the increase of these levels does not pose a danger to either the woman or the fetus during a healthy normal pregnancy unless the levels reach a certain threshold.*

APPLICATION TO PATIENT CARE

- *While increased levels of both ACA and β_2GP IgG antibodies may be a normal physiological phenomenon during pregnancy, the factors that lead autoantibodies to become pathogenic are currently obscure. In view of this, the authors of this study support the current autoantibody measurement practices during pregnancy.*

Antiphospholipid antibodies (APLAs) have been detected in up to 5% of healthy Omani individuals.[1] In a prospective study by Lynch *et al.*, these autoantibodies were found to include the antibodies countering cardiolipin and β_2-glycoprotein (β_2GP), which are directed against phospholipid-binding proteins.[2] Furthermore, a study by Harris *et al.* indicated that anti-cardiolipin antibodies (ACAs) were associated with recurrent pregnancy loss.[3] These studies have also documented the presence of ACAs in pregnant women as a valuable indicator for recurrent abortions and fetal wastage, both in patients with autoimmune diseases and in those with no apparent autoimmune diseases.[1–3] Other studies, however, have not confirmed the significance of the presence of ACAs during pregnancy.[4–7] The changes in APLA levels during pregnancy remain obscure.[8–16] In an early study based on recurrent miscarriages, it was discovered that approximately 20% of the studied women had persistent APLAs before conception.[17] In some cases, a rise in the ACA titre has been noted during early pregnancy.[11–16]

Pregnancy is known to be associated with a T helper-2 (Th2) environment which is thought to enable the maintenance of a normal healthy pregnancy. However, the real mechanisms of and reasons for the shift towards a Th2 profile have yet to be elucidated.[17,18] This study aimed to detect the levels of both immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies specific for both cardiolipin and β_2GP during and after a healthy normal pregnancy.

Methods

This study was conducted between May 2010 and December 2012 and included a total of 150 Omani women. Of this group, 75 were healthy pregnant women who attended the antenatal care outpatient clinic of the Department of Obstetrics & Gynaecology at the Armed Forces Hospital in Muscat, Oman. Healthy non-pregnant women (n = 75) were recruited from the Sultan Qaboos University Hospital blood bank and acted as the control group; they were investigated in parallel with the pregnant women. In both groups, women with the following conditions were excluded from the study: diagnosed connective tissue disease or other autoimmune diseases, or a previous history of thromboembolisms, recurrent abortions or treatments affecting the immune response, such as corticosteroids, immunosuppressive drugs or immunomodulators. The pregnant women were followed up for these conditions throughout their pregnancy and after delivery.

Blood samples were collected from the control group (never pregnant women) and from the pregnant women, both during their first trimester and 90 days after delivery. The sera were separated after centrifugation at 4,000 rpm in a cooling centrifuge. All sera were tested for ACA (using IgM and IgG) and β_2GP levels using a standard enzyme-linked immunosorbent assay (EUROIMMUN Corp., Lübeck, Germany), following the manufacturer's recommendations. The results were expressed in relative units per millilitre (RU/mL). In order to fulfil the Sapporo criteria for antiphospholipid syndrome, autoantibodies were tested twice, with an interval of six weeks between the tests, to distinguish the levels of the antibody response.

Collected data were then analysed using various statistical tests. A Wilcoxon signed-rank test was performed to assess the significance of the changes in antibody levels during pregnancy and after delivery. Furthermore, to assess the significance of such changes, the levels of autoantibodies obtained from the pregnant women during pregnancy and after delivery were compared with the control group of non-pregnant women using the Mann-Whitney U test. The significance of the correlations was calculated using Spearman's rank correlation coefficient. Differences in antibody levels were considered significant at $P <0.05$.

Ethical approval for this study was obtained from the Medical Research & Ethics Committee of the College of Medicine & Health Sciences at Sultan Qaboos University in Muscat, Oman (MREC #654). All of the women involved in the study gave signed informed consent before participation.
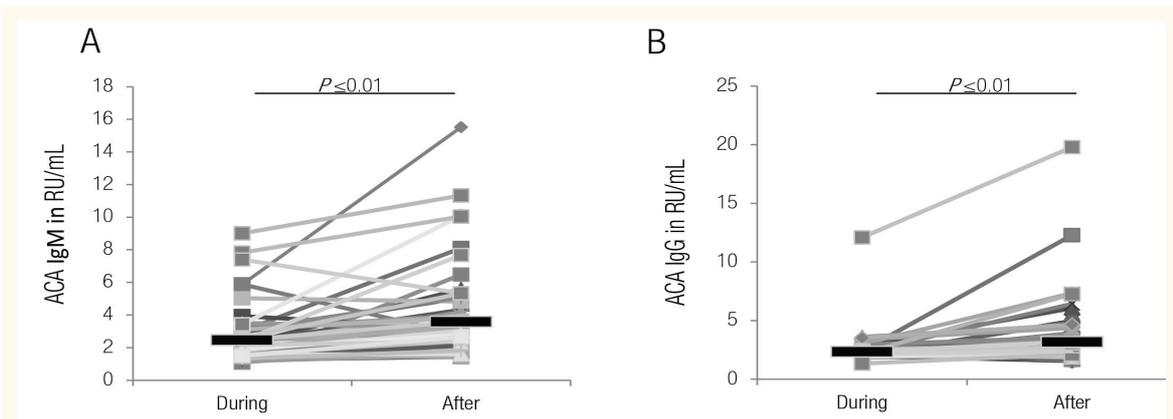
Figure 1 A & B: Increased levels of anti-cardiolipin antibody **(A)** immunoglobulin M and **(B)** immunoglobulin G were observed among the pregnant group of women after delivery in comparison to those recorded during their pregnancies (n = 75).

ACA = anti-cardiolipin antibody; IgM = immunoglobulin M; IgG = immunoglobulin G; RU/mL = relative units per millilitre.

Results

A total of 150 healthy Omani women were investigated, with 75 pregnant women and 75 controls. The mean ages of the pregnant women and the control group were 24 and 27 years, respectively. All investigated pregnant women had uncomplicated pregnancies and normal pregnancy outcomes. Approximately 80% of the pregnant women had been pregnant before their current pregnancies.

Increased levels of IgG were associated with a transient decrease of ACA IgM levels during pregnancy. The average IgM level after delivery (3.5 RU/mL) was significantly higher than that during pregnancy (2.6 RU/mL; $P \leq 0.01$) [Figure 1A]. A similar pattern was found with the serum ACA IgG, with an average of 3.2 RU/mL after delivery in comparison to an average of 2.4 RU/mL during pregnancy ($P \leq 0.01$) [Figure 1B].

The average ACA IgM level was significantly higher in the control group (3.98 RU/mL) than in the pregnant women during their pregnancies ($P < 0.001$) [Figure 2A]. However, no significant differences were seen in the ACA IgM levels between the control group and the pregnant women after delivery. In contrast, ACA IgG levels were significantly higher during and after pregnancy when compared to those of the healthy control group, with an average of 1.8 RU/mL ($P = 0.007$ and 0.002, respectively) [Figure 2B].

Among the pregnant women, a transient increase in the levels of β₂GP IgG was associated with a transient decrease of ACA IgM levels during pregnancy. The average IgM level after delivery (8.4 RU/mL) was significantly higher than the average during pregnancy (2.2 RU/mL; $P \leq 0.01$) [Figure 3A]. In addition, a decrease was observed in the average level of β₂GP IgG after delivery (1.4 RU/mL) in contrast to the average level during pregnancy (2.0 RU/mL; $P \leq 0.01$) [Figure 3B].
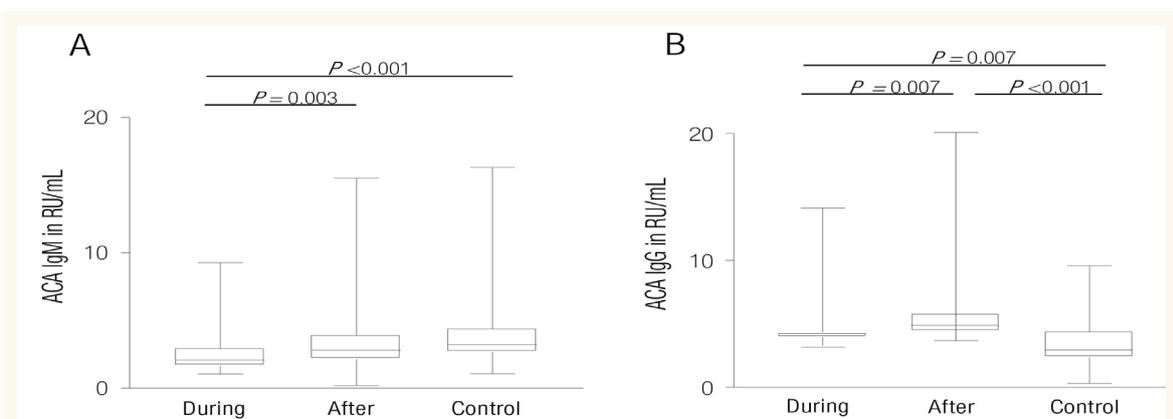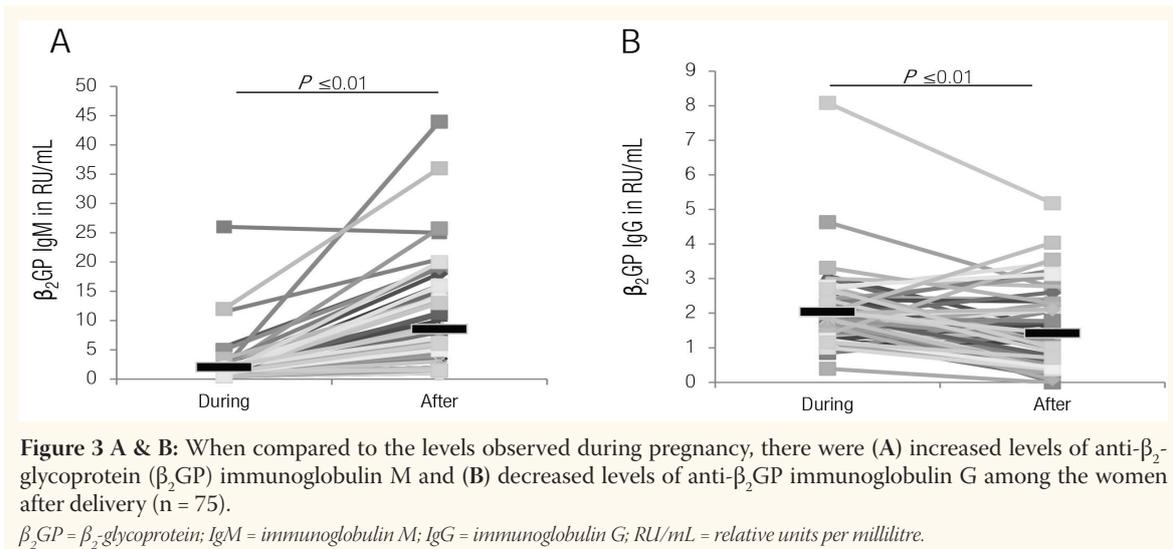
The average level of β₂GP IgM was significantly

Figure 2 A & B: In comparison to the control group (n = 75), there were **(A)** decreased levels of anti-cardiolipin antibody (ACA) immunoglobulin M and **(B)** increased levels of ACA immunoglobulin G among the pregnant women (n = 75), both during pregnancy and after delivery.

ACA = anti-cardiolipin antibody; IgM = immunoglobulin M; IgG = immunoglobulin G; RU/mL = relative units per millilitre.
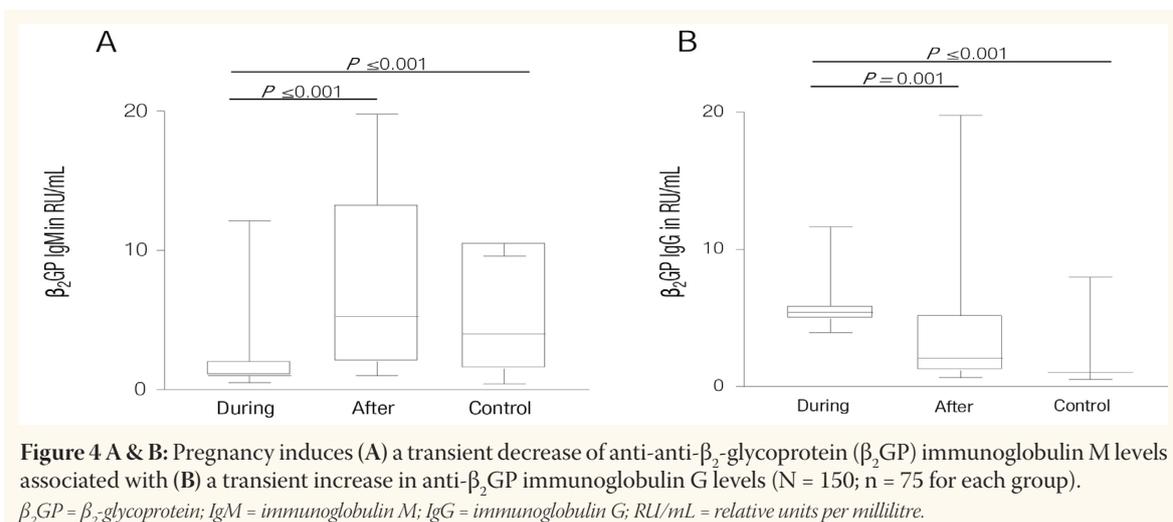
Mohammed S. Al-Balushi, Sidgi S. Hasson, Elias A. Said, Juma Z. Al-Busaidi, Muna S. Al-Daihani, Mohammed S. Othman, Talal A. Sallam, Mohammed A. Idris, Moza Al-Kalbani, Nicholas Woodhouse and Ali A. Al-Jabri

Figure 3 A & B: When compared to the levels observed during pregnancy, there were (**A**) increased levels of anti-β_2-glycoprotein (β_2GP) immunoglobulin M and (**B**) decreased levels of anti-β_2GP immunoglobulin G among the women after delivery (n = 75).

β_2GP = β_2-glycoprotein; IgM = immunoglobulin M; IgG = immunoglobulin G; RU/mL = relative units per millilitre.

lower during pregnancy in comparison to after delivery and in the control group (8.9 RU/mL; P <0.01 and <0.01, respectively) [Figure 4A]. However, no significant differences were observed in the ACA IgM levels during pregnancy in comparison to those recorded after delivery or among the control group.

The levels of β_2GP IgG were found to be significantly higher during pregnancy in contrast to the levels recorded after delivery and in the control group (1.2 RU/mL; P = 0.001 and <0.001, respectively) [Figure 4B].

The levels of both ACA IgG and β_2GP IgG were significantly higher in the pregnant women than in the healthy non-pregnant control group, by 1.3 and 1.6 times, respectively. Moreover, as the pregnant women's β_2GP IgG decreased after delivery to levels similar to those found in non-pregnant women, the ACA IgG levels were still 1.8 times higher after delivery when compared with the control group. Interestingly, the increase of IgG levels during pregnancy was associated

with a transient 40% and 80% decrease in IgM levels against both ACA and β_2GP, respectively [Figures 5A & B].

There were similarities in the fluctuation pattern of ACA and β_2GP autoantibodies in healthy pregnant women. While the results obtained showed that the levels of both ACA IgG and β_2GP IgG were significantly correlated during pregnancy (ρ = 0.3; P = 0.008) [Figure 6A], no correlation was detected between the level of these autoantibodies after delivery and in the control group. Furthermore, the IgM levels of both ACA and β_2GP were significantly correlated during pregnancy (ρ = 0.4; P <0.001) [Figure 6B], after delivery (ρ = 0.5; P <0.001) [Figure 6C] and in the control group (ρ = 0.4; P <0.001) [Figure 6D]. The ratio of IgG ACAs to IgM ACAs was significantly correlated with the IgG to IgM ratio for β_2GP during pregnancy (ρ = 0.5; P <0.001) [Figure 6E] and after delivery (ρ = 0.34; P = 0.004) [Figure 6F].
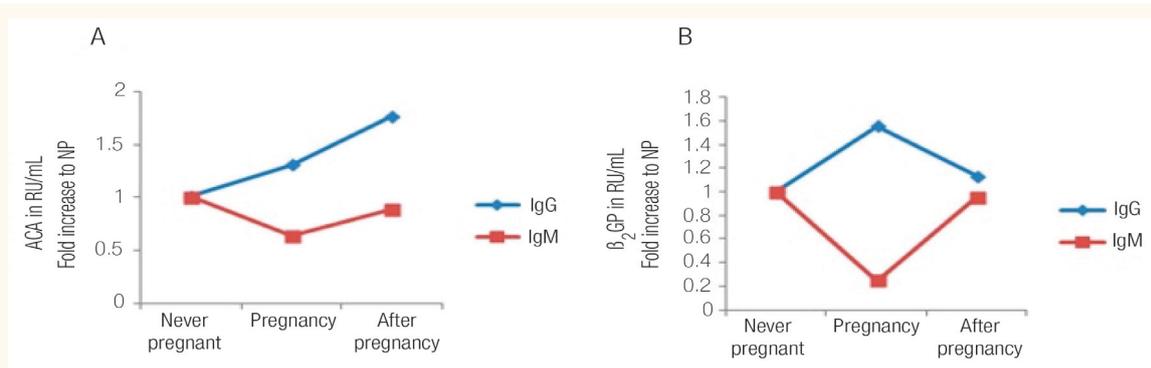

Figure 4 A & B: Pregnancy induces (**A**) a transient decrease of anti-anti-β_2-glycoprotein (β_2GP) immunoglobulin M levels associated with (**B**) a transient increase in anti-β_2GP immunoglobulin G levels (N = 150; n = 75 for each group).

β_2GP = β_2-glycoprotein; IgM = immunoglobulin M; IgG = immunoglobulin G; RU/mL = relative units per millilitre.

Figure 5 A & B: Increased anti-cardiolipin antibody (ACA) and β_2-glycoprotein (β_2GP) immunoglobulin G (IgG) levels were observed during pregnancy (n = 75) in comparison to the never pregnant control group (n = 75). This was associated with a transient decrease in immunoglobulin M (IgM) levels directed against these self-antigens. **A:** Fold increase of ACA IgM and IgG levels. **B:** Fold increase of anti-β_2GP IgM and IgG levels. The values here are calculated based on the mean values of the previous figures.

ACA = anti-cardiolipin antibody; β_2GP = β_2-glycoprotein; IgG = immunoglobulin G; IgM = immunoglobulin M; RU/mL = relative units per millilitre; Np = never pregnant.
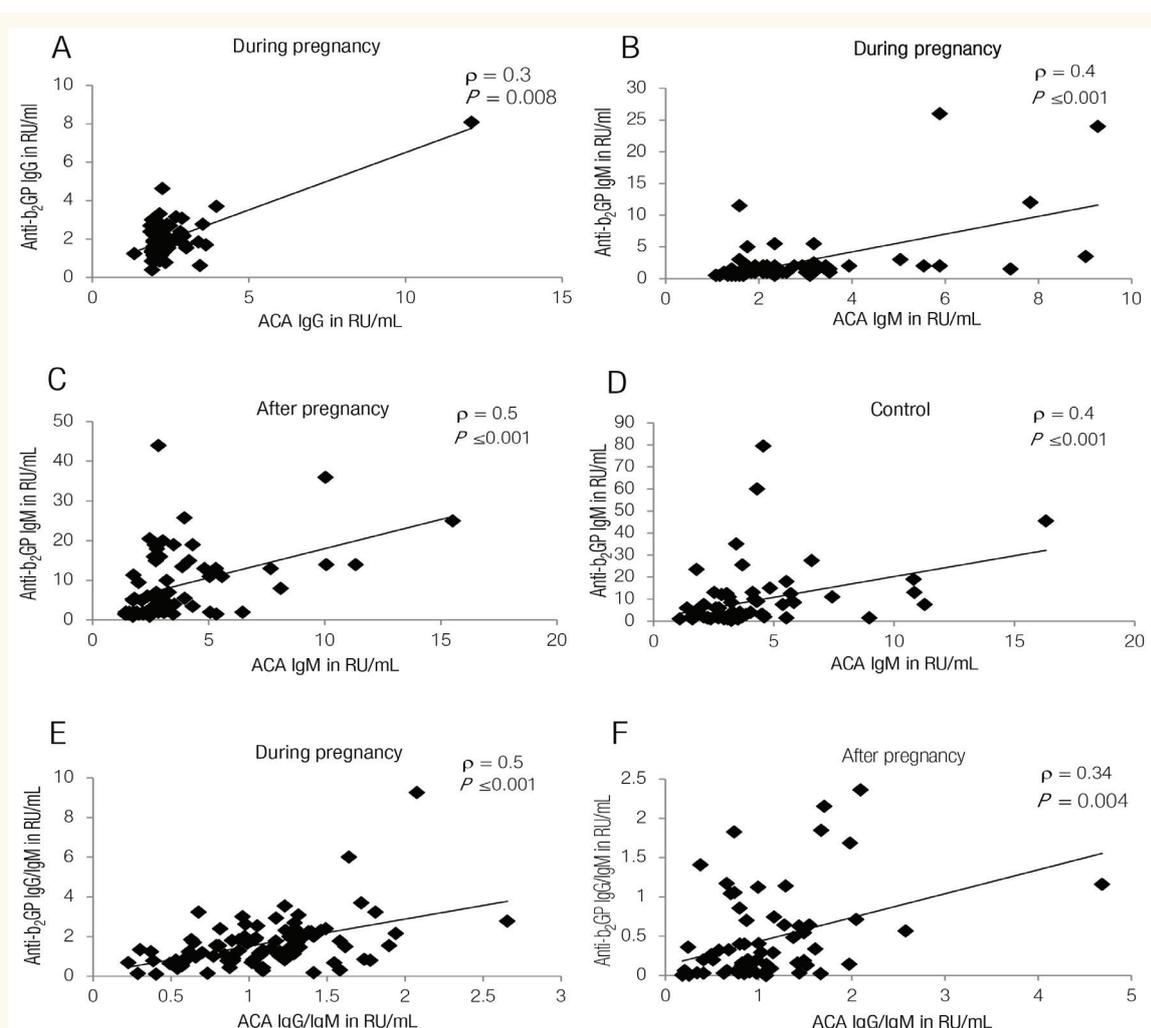

Figure 6 A–F: A correlation was found between anti-cardiolipin antibody (ACA) and anti-β_2-glycoprotein (anti-β_2GP) autoantibodies and (**A**) immunoglobulin G (IgG) levels. Additionally, correlations were found between ACA and anti-β_2GP autoantibodies and immunoglobulin M (IgM) levels (**B**) during pregnancy, (**C**) after delivery and in (**D**) non-pregnant women, respectively. Correlations were also found between the ratios of IgG to IgM for ACA and anti-β_2GP both (**E**) during pregnancy and (**F**) after a normal delivery (N = 150; n = 75 for each group).

ACA = anti-cardiolipin antibody; β_2GP = β_2-glycoprotein; IgG = immunoglobulin G; IgM = immunoglobulin M; RU/mL = relative units per millilitre.

Mohammed S. Al-Balushi, Sidgi S. Hasson, Elias A. Said, Juma Z. Al-Busaidi, Muna S. Al-Daihani, Mohammed S. Othman, Talal A. Sallam, Mohammed A. Idris, Moza Al-Kalbani, Nicholas Woodhouse and Ali A. Al-Jabri

Discussion

The results of this study show that levels of ACA and β_2GP IgG were significantly higher during pregnancy when compared with a non-pregnant control group. Furthermore, although the β_2GP IgG levels of the pregnant women decreased after delivery, their ACA IgG levels remained high. The increase of these IgG levels during pregnancy was associated with a transient decrease in IgM levels. Although these autoantibodies did not reach pathogenic levels, such results suggest that pregnancy promotes the production of these autoantibodies. The presence of autoantibodies at non-pathogenic levels is thought to be important in maintaining homeostasis as they may help the body eliminate the self-structures resulting from cellular or tissue damage.[19] Levels of these autoantibodies may therefore assist in the avoidance of the accumulation of cellular debris that results from cell death, including the apoptotic process. The potential role of these autoantibodies in tumour surveillance, however, needs to be further investigated.[20]

The changes in IgG and IgM levels observed in this study could be due to the Th2 cytokine environment that is associated with and thought to enable the maintenance of a normal healthy pregnancy.[14] However, the exact mechanisms of and reasons for the shift towards a Th2 cytokine profile remain elusive, although hormonal changes have been suggested as a possible cause.[21] The inhibition of the T-helper 1 (Th1) immune response might be important for the survival of the fetus. In fact, while the Th1 response is a pro-inflammatory response, the Th2 response has a tolerogenic effect. It is well established that isotype switching from IgM to certain subclasses of IgG (such as IgG1, the predominant IgG subclass) is a Th2-dependent phenomenon.[15] Therefore, the Th2 profile during a normal healthy pregnancy might be related to the increase of self-recognising IgM and IgG levels. Of note, no significant differences were found in the antibody levels of IgM and IgG specific for ACAs and β_2GPs between women who had been pregnant before and those who were pregnant for the first time.

Interestingly, the results obtained in this study showed that ACA and β_2GP IgG levels were significantly correlated with each other only during pregnancy. This may also be explained by the Th2 cytokine environment believed to occur during a normal healthy pregnancy.[16] This theory is supported by the present study's results, which found significant correlations between the ratio of IgG to IgM levels for both ACAs and β_2GPs. Moreover, another explanation for such a correlation is that the fifth domain of β_2GP contains a phospholipid-binding site (Cys281-Cys288)

and a region recognised by ACAs.[17,18] Consequently, when the β_2GP antigen is available, it might elicit autoantibodies that recognise both ACAs and β_2GPs at the same time. Furthermore, and unlike IgG levels, ACA and β_2GP IgM levels were significantly correlated in the pregnant group during pregnancy and after delivery, as well as in the control group. The presence of such a correlation (with reference to the levels of IgM but not IgG in non-pregnant women) can be explained by the induction of this isotype at a basal level, for reasons that remain elusive. The observed correlation of ACA and β_2GP IgM levels during pregnancy and after delivery might be due to the similarities that are found in the patterns of fluctuation for IgM levels, which is not the case for IgG levels after delivery.

The results of this study clearly showed the correlation between ACA and β_2GP autoantibodies, corroborating previous findings that illustrated similar correlations among pregnant women who experienced spontaneous abortions or pre-eclampsia.[22,23] It is worth noting that the correlation between ACA and β_2GP IgG levels was also found in some autoimmune diseases, such as systemic lupus erythematosus and antiphospholipid syndrome, as well as in some infectious diseases, including human immunodeficiency virus infection, leprosy and malaria.[24–27] The increase in ACA and β_2GP IgG levels occurs even during a healthy pregnancy; however, this increase does not present a threat to the maintenance of the pregnancy unless these autoantibodies reach a certain threshold.[28] The mechanisms that lead to such a pathogenic effect have yet to be confirmed.

In general, autoantibodies have a negative effect on the maintenance of a healthy pregnancy. However, the relationship between ACA levels and recurrent pregnancy loss remains controversial.[1–5] For example, a decrease in the concentration of APLAs has been associated with a successful pregnancy outcome in some studies, whereas others have shown that the relationship between ACA levels and pregnancy loss is not significant at all.[29–32] Consequently, the present study's results on the fluctuation of ACA levels during a normal pregnancy may explain the discrepancies reported by different research groups, as the presence of these autoantibodies did not have a negative influence on the pregnancy, most likely because they were at non-pathogenic levels. Finally, it is interesting that the ACA IgG levels observed in this study increased even after delivery, while β_2GP IgG levels declined to normal levels.

The results of the current study reflect the increase of specific autoantibodies during pregnancy. However, this increase might be general to different autoantibodies, as it is very likely a result of the

particular immunological environment associated with pregnancy. Further studies should be performed on a larger sample size and should investigate a greater number of autoantibodies, such as anti-thyroid peroxidase antibodies, anti-nuclear antibodies and anti-double stranded deoxyribonucleic acid.

Conclusion

Among these samples of healthy pregnant women, ACA and β_2GP autoantibodies were found to increase to levels that do not present a threat to the maintenance of a healthy normal pregnancy. This phenomenon could be due to an increase in isotype switching, as seen by the decrease of IgM levels. The factors that lead autoantibodies to become pathogenic are currently unknown.

ACKNOWLEDGEMENTS

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