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Anti ß$_2$-Glycoprotein I Antibodies in Women with Recurrent Spontaneous Abortion

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Abstract: Problem statement: Antiphospholipid antibodies (aPL) are associated with repeated miscarriages and pregnancy complications, however their pathogenic mechanisms are still matter of research. Anti-β_2 glycoprotein 1 (β_2GPI) antibody is one of these aPL that its contribution to RSA risk remains poorly understood. There were not any data about role this auto-antibody in RSA in South of Iran. This study was conducted to assess Anti- β_2GPI IgM and IgG as RSA risk factors for RSA in this area of Iran. **Approach:** This case-control study was carried out in gynecology special hospital of Hormozgan University of medical sciences, during 2004-2005. A number of 250 women with >3 consecutive idiopathic pregnancy losses as a case group and 200 age matched women with normal full term delivery and negative history of miscarriage as a controls were studied. All obtained sera from the case and the control groups were tested using an Enzyme Linked Immunosorbent Assay (ELISA) method for detection of anti-β_2GPI IgM and IgG. Data was analyzed, using SAS 8 statistical software (chi square and t-test). **Results:** There were significant differences between the prevalence of positive anti-β_2GPI IgM ($p = 0.01$) and IgG ($p = 0.02$) in the case group and the control group. A positive significant relationship was observed in the case group between number of abortion and seropositivity for anti-β_2GPI IgG ($p = 0.028$, $r = +0.181$) and anti-β_2GPI IgM ($p = 0.0381$, $r = +0.014$). **Conclusion:** This study showed anti-ß$_2$GPI antibodies are important causative agent for RSA in this area of Iran.

Key words: Anti-β_2 Glycoprotein-I (β_2GPI) antibody, recurrent spontaneous abortion, etiologic factor, Enzyme Linked Immunosorbent Assay (ELISA), full-term delivery, antiphospholipid antibodies (aPL), thrombosis

INTRODUCTION

The incidence of habitual abortion is in the range of 0.4-0.8% and in approximately half of these cases a specific etiologic factor can be found (Jeon *et al*., 2009). About 3-5% of habitual abortion cases are thought to be due to autoimmune abnormalities (Pandey *et al*., 2005).

Antiphospholipid antibodies (aPL) in addition to cardiovascular diseases (Jahromi *et al*., 2010a; 2010b) are associated with repeated miscarriages and pregnancy complications, however their pathogenic mechanisms are still matter of investigation. Thrombosis at the placental level cannot elucidate all of the clinical manifestations (Meroni *et al*., 2010). It has

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been suggested that aPL may be responsible for a local acute inflammatory response mediated by complement activation (Sotoudeh Jahromi, 2007a; Meroni *et al*., 2010) and neutrophil infiltration eventually leading to fetal loss (Meroni *et al*., 2010). In addition to the Classical Lupus Anticoagulant (LAC) and Anti-Cardiolipin Antibodies (ACA), other anti-Phospholipid Antibodies (aPL) were shown to target anionic phospholipids and other plasma proteins, including phosphatidylethanolamine, protein C, protein S, ß$_2$-Glycoprotein I (GPI) and annexin V (Tincani *et al*., 2010). In spite of their presence, the accurate mechanism by which aPLs precipitate fetal loss is unclear, but may include inducing prothrombotic events in the uteroplacental vasculature (Matsubayashi *et al*., 2001). Beta-2-Glycoprotein I (ß$_2$-GPI), a 50 kDa glycoprotein expressed by Syncytiotrophoblasts (STB) and fetal villi connective tissues 7-weeks post-gestation and targeted by aPL (Loncar, 2010), was implicated in RSA pathogenesis as reduction in its expression by STB was found in women with Antiphospholipid Syndrome (APS) (Miyakis *et al*., 2004). Antibodies against ß$_2$-GPI (anti-β_2GPI) were described as prothrombotic in subjects with APS (Pandey *et al*., 2005) and were recommended to have a role in Recurrent Spontaneous Abortion (RSA), since they inhibited trophoblast proliferation and enhanced thrombosis and abnormal placentation (Norwitz *et al*., 2001).

Insofar as the exact role and clinical significance of anti-ß$_2$GPI antibodies in RSA pathogenesis is doubtful, this study is an effort to address the prevalence of anti-ß$_2$GPI antibodies in women with idiopathic RSA in south of IRAN by the traditional Enzyme-Linked Immunosorbent Assay (ELISA) method.

MATERIALS AND METHODS

We conducted this case-control research in the delivery population of University gynecology Hospital (Hormozgan province, IRAN) between Feb. 2004-Jun. 2005, to investigate whether prevalence of anti-β_2GPI antibodies (IgM and IgG) in two groups of women with RSA as case group and healthy women with successful delivery and without history of abortion as control group to determine a relation between the role of anti-β_2GPI antibodies and RSA.

The cases were 250 women who were recognized with RSA by gynecologist during the study period and control group consisted of 200 asymptomatic women with no history of abortion and full term delivery who were referred to Hormozgan University Hospital.

All subjects gave written consent for obtaining their blood samples according to research purposes. Whole blood samples were collected from all women in both groups. Serum isolation was done by centrifuging of Whole blood samples at 2000×g for 20 min.

The cases were interviewed at their first visit using a standard maternal questionnaire by trained interviewers. Questions were asked about the following: age, parity, gynecologic and medical history of abortion.

Serum anti-ß$_2$GPI IgG and IgM levels were measured by quantitative ELISA, using the *Aeskulisa* kit (Ref, 3206) according to manufacturer's instructions.

Results <19 U mL^{-1} were interpreted as negative, while specimens with values ≥19 U mL^{-1} were considered positive.

All data were analyzed using SAS 8 Statistical Software (SAS Institute, Inc., Cary, NC).

Our study was conducted after all institutional ethics rations were met.

RESULTS

The average age of participants was 25.6±7.6 and 25.3±6.5 years in the case and in the control group respectively. There was no significant difference between age in case and control group ($p = 0.50$). The number of abortion in case group was 5±1.24.

From the sample, 32 (12.8%) women with abortion were seropositive for anti-β_2GPI IgG comparing to 5 (2.5%) women in control group, the difference was significant ($p = 0.02$).

However, only 15 (6.0%) patients were seropositive for anti-β_2GPI IgM in case group when 3 (1.5%) seropositive samples were recognized in control group. The difference between case and control group according to seropositivity for IgM was significant ($p = 0.01$).

A positive significant relationship was observed in the case group between number of abortion and seropositivity for anti-β_2GPI IgG ($p = 0.028$, $r = +0.181$) and anti-β_2GPI IgM ($p = 0.0381$, $r = +0.014$). Also there was a positive significant relationship was observed in the case group between number of pregnancy and seropositivity for anti-β_2GPI IgG ($p = 0.037$, $r = +0.121$) and at the same time no relationship was seen between number of pregnancies and seropositivity for anti-β_2GPI IgM ($p = 0.314$, $r = +0.024$).

We found a positive significant relationship in the control group between number of pregnancy and seropositivity for anti-β_2GPI IgG ($p = 0.041$, $r = +0.103$) and we did not this relationship between number of pregnancies and seropositivity for anti-β_2GPI IgM ($p = 0.591$, $r = +0.018$).

DISCUSSION

Dissimilar the established role of ACA and LAC, the role of anti-ß$_2$GPI antibodies in idiopathic RSA should be more explored, although it is presumed that it involves precipitation of a prothrombotic status that favors altered placentation, poor fetal circulation and probably induction of apoptosis (Halbmayer *et al*., 2005; Rand *et al*., 2005).

Results from this study showed that anti-ß$_2$GPI Abs was elevated in women with idiopathic RSA. Added to our previous findings on association of ACA with RSA (Jahromi *et al*., 2002), results presented here indicate that the presence of these autoantibodies was associated with RSA, via a mechanism which requires participation of immune and also non-immune factors, which in turn precipitate a prothrombotic state that favors fetal loss.

This study indicates that anti-ß$_2$GPI IgM and IgG are a significant risk factor for RSA. Data analysis confirmed a positive relationship between anti-ß$_2$GPI IgM and IgG with number of abortion and number of pregnancy in the case group and a positive relationship between anti-ß$_2$GPI IgG with number of pregnancy in the control group.

Results on the role of anti-ß$_2$GPI on RSA are controversial and some reports described an association of anti-ß$_2$GPI IgG with RSA (Chauleur *et al*., 2010; Zammiti *et al*., 2006; Alijotas-Reig *et al*., 2010; Galli *et al*., 1990) and others implicated IgM (Forastiero *et al*., 1997), but not IgG (Opatrny *et al*., 2006; Jahromi *et al*., 2010) anti-ß$_2$GPI, as possible risk factor for idiopathic RSA, as also shown here. Previous studies showed that no important association of anti-ß$_2$GPI antibodies in women with three or more repeated pregnancy losses (Opatrny *et al*., 2006) and in antiphospholipid-positive patients with obstetric complications (two or more spontaneous fetal losses) (Forastiero *et al*., 1997).

This may be explained by the differences in test sensitivity and in the low number of patients included in some studies (Forastiero *et al*., 1997).

Previous studies about etiologic factors for abortion in this area of IRAN (Bandarabbas), infectious agents such as *Listeria monocytogenes* (Jamshidi *et al*., 2009), *Toxoplasma gondii* (Sotoudeh Jahromi, 2007b) and Cytomegalovirus (Jahromi *et al*., 2010a), *Chlamydia tracomatis* (Jahromi *et al*., 2010b) and also immunologic factor such as ACA and antinuclear antibody (Jahromi *et al*., 2002) were suggested as important causative agents for spontaneous abortion.

In some countries socio-economic and demographic factors (Alpu and Kurt, 2004) dengue virus (Alvarenga *et al*., 2009) and women age and their parity (Adeleke and Adepoju, 2010) were suggested as cause of abortion.

CONCLUSION

Our results suggest that anti-ß$_2$GPI antibodies are related to RSA and should be considered as a pregnancy-loss risk factor. As this antibody is categorized in antiphosohlipid antibodies (Tincani *et al*., 2010) and also ACA was suggested as risk factor for RSA in the same area (Jahromi *et al*., 2002), it may be other antiphosohlipid antibodies are participating in RSA in this area.

Thus it is recommended further research to explore the role of other antiphosohlipid antibodies in RSA in this area and also all patients with RSA must be evaluate for antiphosohlipid syndrome for appropriate treatment.

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