

Correlation between Antiphospholipid antibodies and Recurrent Spontaneous Miscarriage in Saudi Women

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Abstract: ***Objective:** To examine the association between antiphospholipid antibodies and gestational age of abortion and duration post abortion in Saudi women living in Western region of Saudi Arabia (Makkah) in order to consider the interactive association with major demographic factors. **Materials and Methods:** In this free-living population study blood samples were collected from 100 females who had history of spontaneous recurrent abortion as case group and 100 pregnant healthy women as control group ages ranged between 22 and 40 years, with a mean age of 31.4 ± 12.69 years. Serum antiphospholipid antibodies were measured. **Results:** Average rate of antiphospholipid antibodies in the serum of case group compared with the control group indicated no significant statistical difference. In case group the number of miscarriages were more, mean of antiphospholipid antibody levels were also higher. Mean antiphospholipid antibodies rate was greater with increasing gestational age at time of first miscarriage. Almost mean antiphospholipid antibodies in all patients remained in high level just in first 4 years with any number of miscarriages and 4 years later, antibodies began to fall. **Conclusion:** The results of the present study demonstrated that antiphospholipid antibodies based on number of miscarriages and gestational age of miscarriages were increased. More over mean antiphospholipid antibodies in all patients remained in high level just in first 4 years post miscarriage and then began to fall. However, well-designed diagnostic studies are needed to estimate the true association between other specific autoantibodies and recurrent miscarriage through epidemiological studies with a larger sample size, including different age groups and populations.*

Keywords: Antiphospholipid antibodies, recurrent spontaneous miscarriage, Saudi Arabia

1. Introduction

Antiphospholipid syndrome (APS) is a group of diseases characterized by recurrent spontaneous abortion (RSA), still birth, premature birth, and serum antiphospholipid antibodies (APL). The incidence of RSA is approximately 2–4%, [1] and 65–70% of RSA cases are complicated by abnormal immune factors[2]. APS is the most common cause of immune-related RSA; nearly 7–25% of RSA patients have APS [3].

The antiphospholipid syndrome was first reported in the early 1980s as the association of thrombosis, recurrent pregnancy loss in the presence of anticardiolipin antibodies (aCL) and/or lupus anticoagulant[4]. Antiphospholipid syndrome is characterized by venous or arterial thrombosis and/or pregnancy loss in the presence of persistent antiphospholipid antibodies (aPL). It can occur as a primary condition, or it can occur with systemic lupus erythematosus (SLE) or another systemic autoimmune disease. Patients with SLE frequently have aPL, whereas the development of APS is much less common. aPL can also be found in healthy individuals. The main types of aPL of concern during pregnancy are lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and anti-beta-2-glycoprotein-1 antibodies [5].

The antiphospholipid syndrome was reported in the early 1980s as the association of thrombosis, recurrent pregnancy loss in the presence of anticardiolipin antibodies and/or lupus anticoagulant. Since then, many other clinical manifestations have been associated with antiphospholipid. Almost any organ and tissue may be involved in the disease, including the brain, the heart, the kidneys, the placenta and

many more. Antiphospholipid syndrome is characterized by recurrent arterial or venous thromboembolism or pregnancy loss in association with antibodies directed against anionic phospholipids or plasma proteins bound to anionic phospholipids. In accordance with this, fetal abortion, typically beyond the tenth week of gestation, is also caused by infarctions of blood vessels in the placenta [6]. Among the autoimmune factors, anti-phospholipid antibodies have been demonstrated to be the strongest risk factors for fetal loss, the prevalence of which is as high as 40% in women with recurrent fetal loss[7]. But the pathophysiologic mechanisms that characterize thrombosis and recurrent pregnancy losses are still not clear[8]. Thrombotic events at the placental level cannot explain all of the clinical manifestations. It has been suggested that anticardiolipin may be responsible for a local acute inflammatory response mediated by complement activation and neutrophil infiltration eventually leading to fetal loss[9]. Obstetric complications such as fetal death, premature delivery, preeclampsia and recurrent abortions are characteristic manifestations of antiphospholipid syndrome [10]. The antiphospholipid antibody syndrome is an autoimmune condition in which vascular thrombosis and/or recurrent pregnancy losses occur in patients [11] and are risk factors for recurrent pregnancy loss and obstetric complications [12] which is characterized by recurrent fetal loss, thrombosis, and thrombocytopenia in association with anticardiolipin antibodies [13].

Although it is established now that APS is characterized by auto antibodies against negatively charged phospholipids in the serum, and clinically by multiple thromboses, thrombocytopenia, and recurrent fetal loss, the mechanism by which the antibodies cause the clinical picture are not

clear and the pathogenesis of this disorder remains poorly understood. For example, it is difficult to predict who will develop APS, or why. Though specific serological characteristics of anti-phospholipid antibodies provide insight into which patients with these antibodies are more likely to develop clinical manifestations, our ability to accurately risk-stratify RSA patients with these antibodies remains limited.

Move over since very limited information is available regarding APS associated pregnancy loss in Saudi population, the current study has been undertaken to evaluate the prevalence of antiphospholipid antibodies among Saudi women with recurrent miscarriage and to determine any association between antiphospholipid antibodies and number of miscarriage and duration past miscarriage.

2. Materials and Methods

This study was done on female inhabitants of Makkah, Saudi Arabia. Subject ages ranged between 22 and 40 years, with a mean age of 31.4 ± 12.69 years, after getting approval by the ethics committee of the affiliated institution. All reference individuals enrolled in this study written informed consent prior to the study. Each candidate was required to complete a physical examination by a certified physician to check the health conditions. Inclusion criteria included history of two or more abortions, no history of medical diseases and drug use. Abortion means fetal loss before 20 weeks of gestational age or fetal weight lower than 500 gram. The exclusion criteria were as followings: presence of acute and chronic infections, digestive diseases, kidney disease, metabolic and nutritional diseases, rheumatic diseases, endocrine disease, circulation system diseases, burns and muscle trauma, hypertension (systolic pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg), excessive smoking (smoking > 20 cigarettes/day), massive blood loss, malnutrition (lose weight, poverty, or special dietary habits) and symptoms (low BMI or significant weight loss), surgery undergone within six months, medication taken within two weeks, blood donation or blood transfusion within four months, strenuous exercise or heavy manual labor. Individuals were further excluded in accordance with one of the following criteria: Positive results for Hepatitis B surface antigen, Hepatitis C antibodies, or HIV antibodies.

100 women with history of miscarriages who had inclusion criteria selected as case group and 100 healthy women without history of miscarriages and had one or more successful pregnancies selected as control group. In the case group history included parity, live birth, number of miscarriages, gestational age at each miscarriage and duration post miscarriage were determined.

About three to five milliliters of blood was drawn. Samples were allowed to clot for half an hour at room temperature, then centrifuged using ALC centrifuge PK130 made in the U.S.A adjusted at 3400 r.p.m. for five minutes. Serum was transferred into sterile serum container for testing. Some of the samples tested were excluded from analysis as they showed abnormal look such as visible hemolysis. Level of antiphospholipid antibodies in samples were measured by

enzyme linked immunoassay test and use of Orgentec kits and relation between mean of antiphospholipid antibodies and number of miscarriage ≥ 3 and more than 4, gestational age at the time of abortions (≤ 10 week, 11–20 weeks, > 20 week) and duration post abortions (≤ 4 year, 5–12 years, > 12 years) were determined. Collected data was analyzed by Student t tests using SPSS program 17.0 (SPSS Institute, Inc.; Chicago, IL, USA) software for statistical analysis. Results were presented as mean \pm standard deviation. P value less than 0.05 was considered significant.

3. Results

Average rate of antiphospholipid antibodies in the serum of case group compared with the control group in this study as shown in figure 1, indicated no significant statistical difference ($p < 0.01$). Estimation of serum antiphospholipid antibodies based on number of previous miscarriages in Saudi women as shown in Figure 2, indicated a significantly high rate ($p < 0.005$) with an increase in the number of miscarriage (four and more).

Based on gestational age in patients of different miscarriages, data for the average distribution of serum antiphospholipid antibodies is presented in Figures 3 A, B & C, respectively. Figure 3A, indicated a consistently high level of serum antiphospholipid antibodies in second and third miscarriages when compared with the first miscarriage at the age of around 10 weeks ($p < 0.050$). Based on gestational age of miscarriages, 11–20 weeks (Figure 3B), and more than 20 weeks (Figure 3C), an increased level of serum antiphospholipid antibodies was observed in the second miscarriage, being highest at the gestational age of 11–20 weeks.

Average distribution of antiphospholipid antibodies based on duration past of different miscarriages is shown in Figures 4 A, B & C. These results indicated a significantly high level at ≤ 4 years after their first miscarriage as compared to second and third miscarriages, being highest around four years than 5–12 years and more than 12 years (Figure 4 A). Moreover estimated levels of serum antiphospholipid antibodies were found to be more or less equal at 5–12 and more than 12 years in all the miscarriages (Figures 4B & C, respectively).

4. Discussion

The pathogenesis of pregnancy morbidity in APS is incompletely understood, but is thought to involve platelet and endothelial cell activation as well as procoagulant effects of antiphospholipid antibodies (aPL)[14].

Although uteroplacental thrombosis and vascular insufficiency may be one mechanism for adverse pregnancy outcome, not all affected placentas display signs of thrombosis or infarction. aPL also appears to have a direct effect on human placental trophoblast function decreasing trophoblast viability, syncytialization, and capacity for invasion as measured by an in vitro assay [15]. In addition, aPL may affect the production of hormones and signaling molecules by cells in the trophoblast, and stimulate coagulation and complement activation [16]. Placental

neutrophil extracellular traps are characteristic of APS dysfunctional placentas, though similar findings characterize placentas from preeclamptic patients [17].

It is unclear whether women with aPL who do not meet criteria for APS are at increased risk of pregnancy morbidity. The body of evidence suggests little or no increase in risk in this group [18]. In a previous study Ghosh [19] evaluated the prevalence of antiphospholipid syndrome among women with recurrent miscarriages/late pregnancy loss showing 27.7% positive for antiphospholipid antibodies. Velayuthaprabhu et al. [20] evaluated the anticardiolipin antibodies and antiphosphatidyl serine antibodies in women with recurrent abortion and concluded that anticardiolipin antibody is found to be the most important factor for recurrent abortion. These results are in conformity with a previous study [21] thus concluding that multiple antiphospholipid specificities in recurrent pregnancy loss group is not significantly different from control group. Antiphospholipid antibodies based on number and gestational age of abortions in our results were found to be increased. Whatever the gestational age was higher in all number of abortion, mean antiphospholipid antibodies was higher. Almost mean antiphospholipid antibodies in all patients were in high level just in first 5 years post all abortions but decreased after more than 5 years.

Although there is no agreement on the mechanisms of recurrent pregnancy loss in patients with these antibodies, vasculopathy of terminal spiral arteries may be implicated and there is a general consensus to routinely screen for antiphospholipid antibodies in patients with recurrent miscarriage believing that the antiphospholipid antibodies seem to be clearly associated with recurrent miscarriage [22]. The risk of first-time thrombosis in pregnant women with aPL and no personal history of thrombosis is still uncertain. Furthermore, there is no strong evidence for an association between aPL and primary infertility, in vitro fertilization failure, or mild or near-term/term preeclampsia [23,24]. Well-designed diagnostic studies are needed to estimate the true association between other specific auto antibodies and recurrent miscarriage.

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Foot Note

This research article is a part of on going research project No: 43509020, Institute of Scientific Research, Umm-Al-Qura University, Makkah, Saudi Arabia.

Figures and Legends

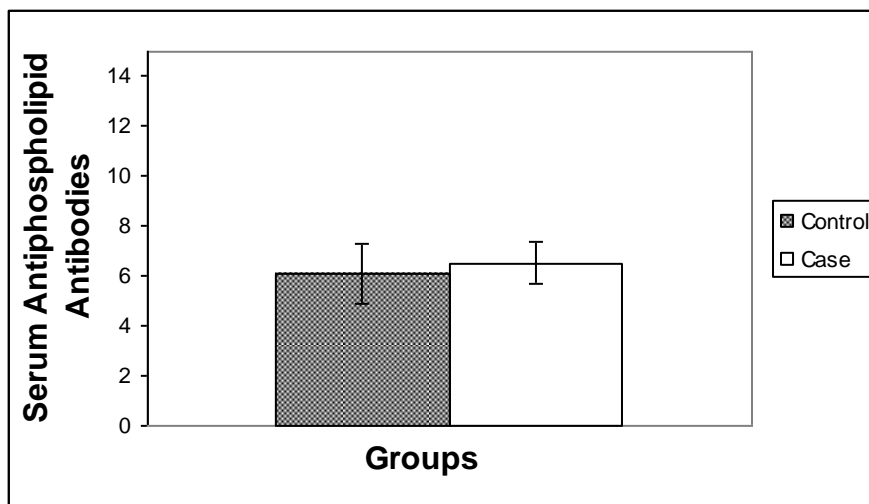


Figure 1: Estimation of serum antiphospholipid antibodies in case group compared with the control group in Saudi women. Values are Mean ± SD, (n = 200).

Legend indicates control and case groups.

Note: n = Total number of subjects examined.

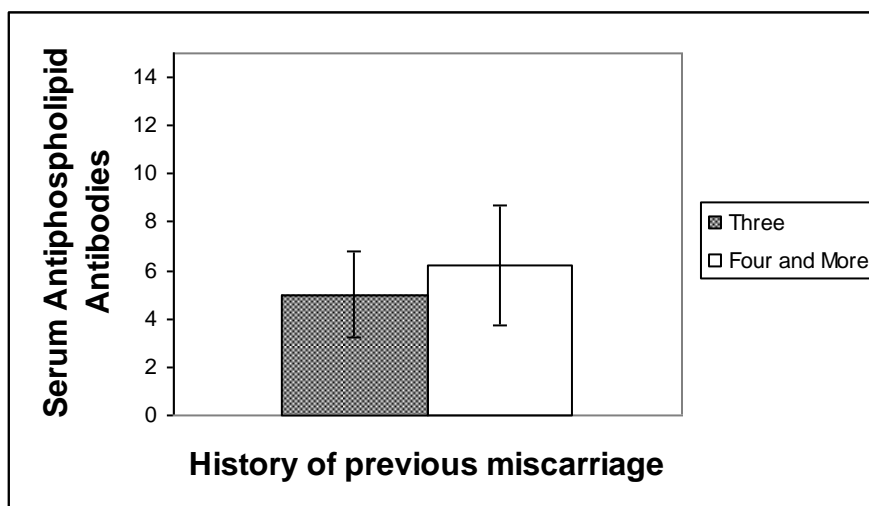


Figure2: Estimation of serum antiphospholipid antibodies based on number of previous miscarriages in Saudi women. Values are Mean ± SD, (n = 100).

Legend indicates number of miscarriages

Note: n = Total number of subjects examined. p<0.05

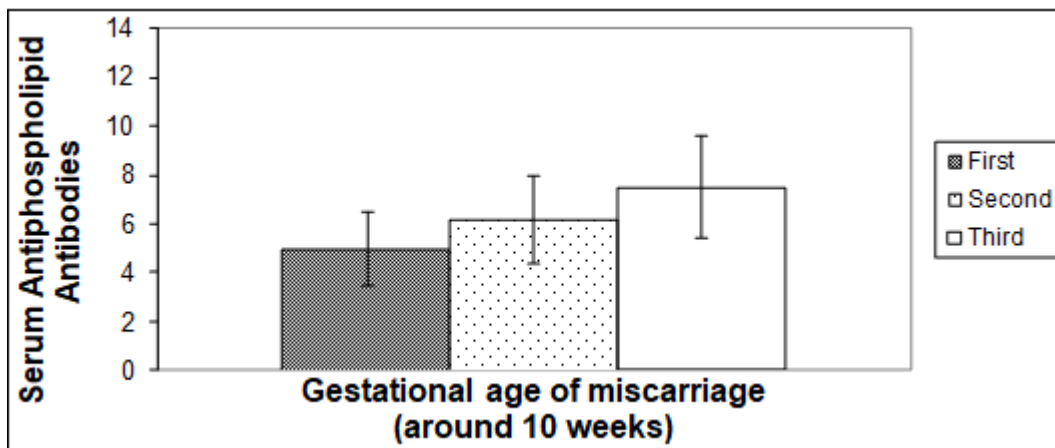


Figure3-A: Estimation of serum antiphospholipid antibodies based on gestational age of miscarriages (≤ 10 weeks in Saudi women. Values are Mean \pm SD, (n = 100).

Legend indicates number of miscarriages

Note: n = Total number of subjects examined. $p < 0.05$

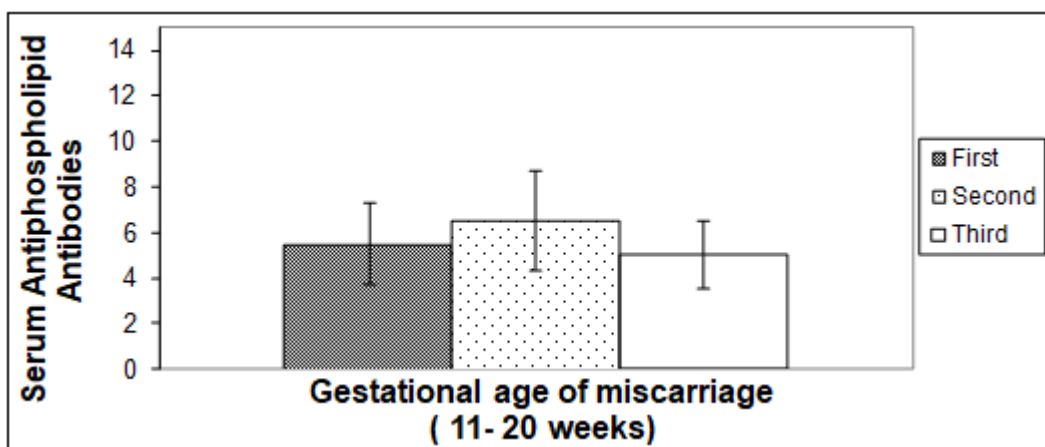


Figure3-B: Estimation of serum antiphospholipid antibodies based on gestational age of miscarriages (11-20 weeks) in Saudi women. Values are Mean \pm SD, (n = 100).

Legend indicates number of miscarriages

Note: n = Total number of subjects examined.

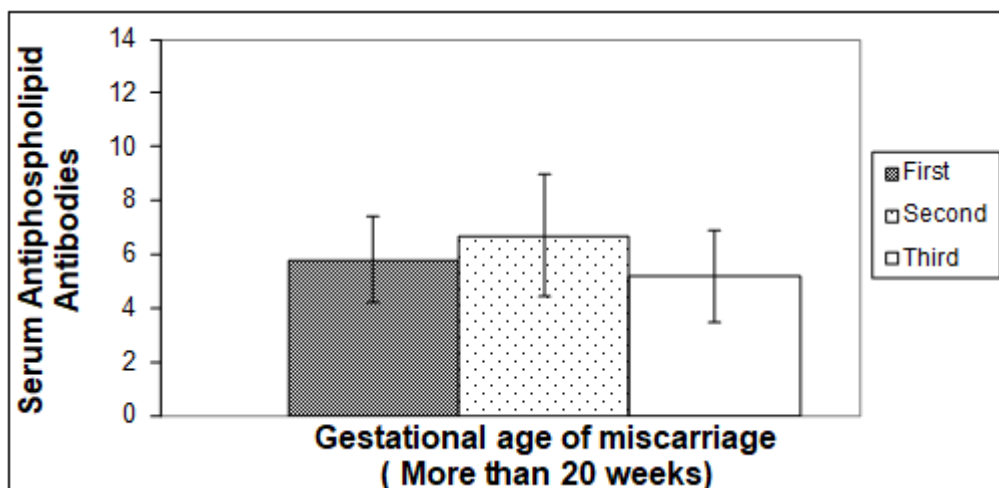


Figure3-C: Estimation of serum antiphospholipid antibodies based on gestational age of miscarriages (More than 20 weeks) in Saudi women. Values are Mean \pm SD, (n = 100).

Legend indicates number of miscarriages

Note: n = Total number of subjects examined.

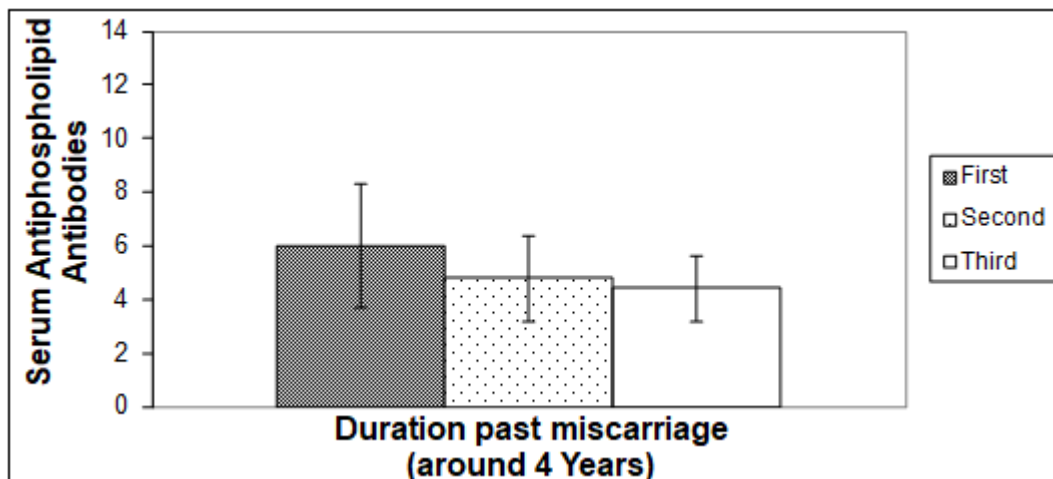


Figure4-A: Estimation of serum antiphospholipid antibodies based on duration past miscarriages (≤ 4 years) in Saudi women. Values are Mean \pm SD, (n = 100).
 Legend indicates number of miscarriages
Note: n = Total number of subjects examined.

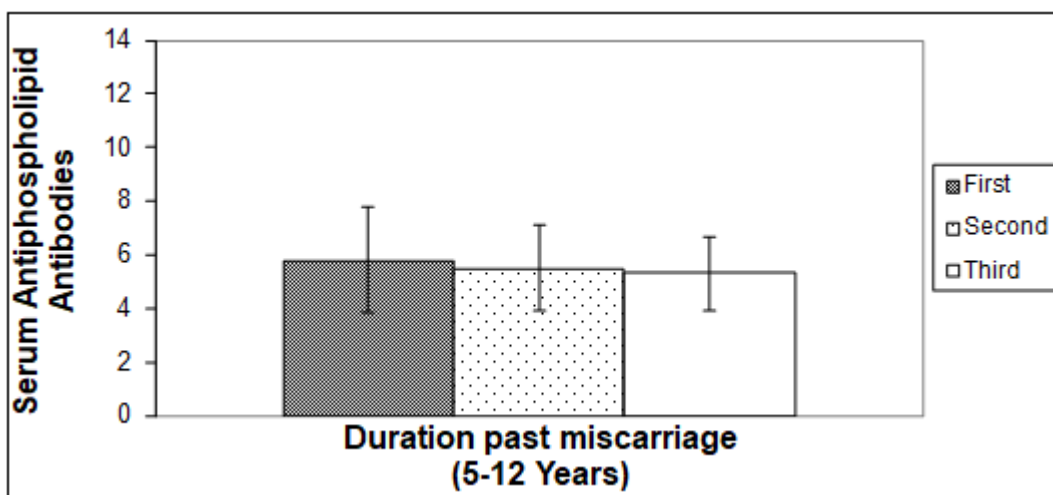


Figure4-B: Estimation of serum antiphospholipid antibodies based on duration post miscarriages (≤ 4 years) in Saudi women. Values are Mean \pm SD, (n = 100).
 Legend indicates number of miscarriages
Note: n = Total number of subjects examined.

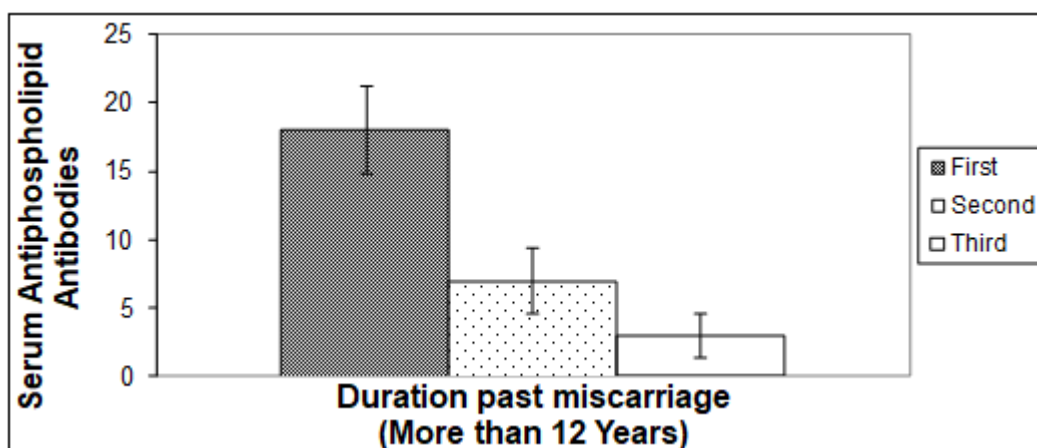


Figure4-C: Estimation of serum antiphospholipid antibodies based on duration past miscarriages (≤ 4 years) in Saudi women. Values are Mean \pm SD, (n = 100).
 Legend indicates number of miscarriages
Note: n = Total number of subjects examined.