Assessment of Serum anticardiolipin and anti bet2-glycoporotin1 among Sudanese women with recurrent miscarriage in Khartoum state - Sudan

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Abstract:

Introduction: Antiphospholipid antibodies (APA) are a heterogeneous family of approximately twenty auto antibodies directed against phospholipids binding plasma proteins

Objective: The aim of the study was to assess of serum anti cardiolipin and serum anti bet2-glycoporotin1 among some Sudanese woman with recurrent miscarriage.

Methodology: This study was conducted in Turkey teaching Hospital, Omdurman maternity hospital and Khartoum north teaching hospital in great state of Khartoum from 2014to 2016. The study was performed on 100 patients as the study group and 100 healthy. The age for the control group and the test group were matched. Serum levels of anticardiolipin (ACL) and serum antibet2-glycoporotin1 (Aβ2GPI) were measured by enzyme-linked immunosorbent assays.

Rustles: There were significant increase in mean of serum anticardiolipin antibodies (IgA, IgG, IgM) ; mean ±SD was (IgA =4.62± 1.9 U/mL), (12.61± 1.7 U/mL), (6.16± 1.5 U/mL) for patients with recurrent miscarriage compared with control group; mean ±SD (3.52± 3.4 U/mL), (1.92± .48 u/mL), (1.77± .82 u/mL) P value (0.006), (0.000), (0.000) respectively. The same significant difference (p=0.000) was observed with the serum antifet2-glycoprotein1 in the study group compared to the control group (10.57± 6.5 vs 3.8± 2.5 ) respectively. There was a significant correlation (r.0.239, P<0.017) between the levels of IgG of ACL and(IgG) anti-beta2GPI antibodies. ACL frequency was reported in 32% of patients with recurrent miscarriage and in 2% of control group.

Conclusions: A significant association was observed between recurrent spontaneous miscarriage and the presence of serum anticardiolipin(IgA,IgG,IgM)and, antifet2-glycoporotin1 (IgG). Also, there was a significant relationship between positive anticardiolipin (Ig G ) and antifet2-glycoporotin1 .

Keywords: anticardiolipin, antifet2-glycoporotin1, recurrent miscarriage
Introduction: Antiphospholipid antibodies (APA) are a heterogeneous family of approximately twenty auto antibodies directed against phospholipids binding plasma proteins. They are Associate with systemic thrombosis including cerebral ischemia, deep vein thrombosis, pulmonary embolism and myocardial infarction. The three most clinically significant are lupus anticoagulant, anticardiolipin antibodies and anti-B2 glycoprotein I antibodies[1].Antiphospholipid syndrome (APS) is one of the known causes of first- and second-trimester recurrent miscarriage. APS is defined as the presence of anticardiolipin antibodies or lupus anticoagulant antibodies, in association with either three or more consecutive fetal losses before week 10 of gestation, one or more unexplained intrauterine deaths beyond 10 weeks of gestation, or one or more premature births before 34 weeks due to severe pre-eclampsia or impaired fetal growth[2].Cardiolipin is a phospholipid found in inner mitochondrial membrane primarily, but it is also a minor constituent of mammalian membranes in general .In diseases with mitochondrial damage, cardiolipin can start an antibody response. Antiphospholipid antibodies are a class of auto antibodies which has been found in 1-5% of systematically healthy population[3]. b2-Glycoprotein I (b2-GPI), a
single-chain 50 kDa glycoprotein and a target of aPL [4], was found to be involved in recurrent spontaneous abortion (RSA), as its reduced expression was reported in women with APS [5]. Anti-bet2-glycoprotein1 (anti-(b2GP1) are antibodies which recognize a plasma protein known as apolipoprotein H or beta-2 glycoprotein I and have higher specificity than anticardiolipin antibodies (ACA) for thrombosis [6]. The mechanism of thrombosis in patients with antiphospholipid antibodies (APA) is still unknown, although several mechanisms have been proposed [7]. There are data suggesting that antiphospholipid antibodies induce thrombosis through any one or more of several mechanisms: (i) antiphospholipid antibody interference with endogenous anticoagulant mechanisms (disruption of the annexin A5 anticoagulant shield [8], inhibition of protein C pathway, inhibition of antithrombin); (ii) binding and activation of platelets; (iii) increased thromboxane production by platelets; (iv) interacting with endothelial cells and inducing expression of adhesion molecules and tissue factor, and thus a prothrombotic state occurs as a result of the reaction between APA with cellular antigens including platelet and endothelial cell membrane protein and (v) decreased prostacyclin production by endothelial cells [9,10].

Recurrent miscarriage is usually defined as three or more concepive, spontaneous miscarriages occurring in the first trimester, with the same biological father [1]. They may or may not follow a successful birth. About half of recurrent miscarriages are unexplained. Recurrent pregnancy loss (RPL) is a major problem affecting 1–2% of women of reproductive age. While Chromosomal aberrations, endocrinological dysfunction, uterine abnormalities are aetiological factors, until recently in most cases, a cause for RPL could not be identified [7]. Gestational outcome in women with inherited thrombophilias who present with RPL is poor with less than 25% of pregnancies resulting in live birth. RPL is a well established finding in women with antiphospholipids syndrome [11]. The aim of this study was to evaluated of serum anti cardiolipin and serum anti bet2-glycoporotin1 among some Sudanese woman with recurrent miscarriage.

Materials and methods:

study Area, and period: This study is gross –sectional was conducted in great Khartoum state Turkey Teaching Hospital, Omdurman Maternity Hospital and Khartoum North Teaching Hospital during the period from February 2014 to February 2016.

Study population: Two hundred `subjects(women), hundred patients diagnosed with two or
more consecutive recurrent miscarriages, were included as the patients and other hundred healthy volunteer women as the control group.

**Data collection.** Data were collected by carefully designed questionnaire. Venous blood sample (5mL) was taken from each participant using disposable syringe. The blood samples were allowed to clot at room temperature and then serum was obtained after centrifugation at 3000 rpm. The clear serum was withdrawn by means of pipette and transferred to plan container and stored at -70°C.

**Sample processing:**

**Measurement of serum Anticardiolipin:** Serum levels of anticardiolipin IgA, IgM & IgG antibodies were measured using EUROIMMUN Medizinsche Labordiagnostika AG (Germany). The procedure is according to the direction of manufacturers[12]. Diluted, samples were transferred into 96 well plates coated with bovine cardiolipin and saturated B2-glycoprotein-1 along with controls. The plates were incubated for 30 minutes at room temperature. Washing was undertaken in triplicate by using washing buffer (PBS, NaN3<0.1%). Enzyme conjugate (Horseradish peroxidase (HRP) conjugated polyclonal rabbit anti-human IgG, IgA, IgM) was added to each well and then incubated for 15 min at RT. Reaction was catalyzed by adding TMB substrate solution to each well and the plate were incubated for 15 minute in dark at room temperature and then washed as before. The reaction was stopped by adding stop solution (1 M hydrochloric acid) [12]; Read O.D by ELISA[13], reader at length of 450 nm. Results were expressed in U/mL with <12 U/mL as Negative result & > 12 U/mL as positive results.

**Measurement of serum Antibet2-glycoprotein1:** Serum anti-b2-GPI IgG and IgM levels were measured by quantitative ELISA, using the EUROIMMUN Medizinsche Labordiagnostika AG (Germany) [12], 12 purified beta2GP was coated on plastic micro well plates and stabilized. All sera was diluted 1:200 with a supplied sample diluent. Next, 100 microliters of the test calibrators, controls and diluted patient samples were added in duplicate to test wells. The wells were covered and incubated for 30 minutes at room temperature. The wells were then washed by adding 300 mcl of buffer to each well. The buffer was flicked out of the wells and the wash repeated three times. A HRP IgG, IgM conjugate was added for 30 minutes followed by another
wash step (300 mcl of wash buffer added to each well, flicked off and repeated three times ). Following this step, 100 mcl of chromogen was added to each well, and the trays were incubated in the dark for 20 minutes. Finally, the stop solution was added and the absorbence read as the mean OD at 450 nm. Results of ,20 RU/ml were interpreted as negative, while specimens with values of > 20 R U/ml were considered positive.

Those patients with positive anticardiolipin antibody or bet2-glycoprotein1 tests were sent to (same laboratory )for another assessment of anticardiolipin level\bet2-glycoprotein1 six weeks later to exclude any accidental elevation in these antibodies in the index patient [14].The mean of the two measurements were taken as the final reading & percentage calculation was carried out.

It is required that the precision of commercially developed assays be evaluated at several levels by the manufacturers, and published in the instruction manuals. The between-run (or total) precision reflects the precision of the assay during routine use (within-run precision is usually too optimistic), so ideally it should be the basis of judging the performance of a test.

Technological advances in the field of automation, manufacturing, and measuring procedures make the above-mentioned goal of 10% CV realistic even for aPL antibody measurements. Developers of commercial and in-house assays should aim for this performance goal. For traditional, manually performed assays, a level of 15% imprecision would still be acceptable[12]

Data Analysis:
The study was approved by the ethical committee of Omdurman Islamic university for clinical and health researches, all the participants were full informed about the aim and benefit of this study. Verbal consent was obtained from all the participants

The data were analyzed using SPSS, version 11,5.the mean and standard deviation of serum level of anticardiolipin and β2-glycoprotein I antibodies were obtained for both patients and control group using t-tests ,multiple comparison s(one way ANOVA test),and Pearson correlation coefficient were used ( P-value< 0.05 was considered significant)

Results:
This is study was take up in 200 subjects (women) their rang of ages were (18-46) years. One hundred women as the patients group. And other hundred healthy women included as the control.
The mean of ages among patients group were 32.32± 6.02 years . The mean of miscarriage (recurrent pregnancy loss) was 3.63 ± 1.25. There was insignificant difference between the mean of the ages in patients group and control group (32.32± 6.02 years versus 32.50± 5.062 ) respectively, (p value 0.8) (table1). The means serum anticardilipin antibodies ( IgA ,IgG ,IgM) ; mean ±SD was (4.62± 1.9 U/mL), (12.61± 1.7 u/mL), (6.16± 1.5 U/mL) for patients with recurrent miscarriage compared with control group; mean ±SD (3.52± 3.4 U/mL), (1.92± .48 u/mL), (1.77± .82 u/mL) This difference was found to be highly significant at P value (0 .004), (0.000), (0.000) respectively(Table 1).

Table 1: Comparison of serum anti cardiolipin and Bet2glycoprotin1(IgA,IgG and IgM) Among study population

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>subjects(n=200)</th>
<th>Anticardiolipin (U/ml)</th>
<th>Bet2glycoprotin1(RU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD</td>
<td>P- values</td>
<td>mean±SD</td>
</tr>
<tr>
<td>IgA</td>
<td>Patients (n= 100)</td>
<td>4.62± 1.9</td>
<td>&lt; 0.004</td>
</tr>
<tr>
<td></td>
<td>Control (n= 100)</td>
<td>3.52± 3.4</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>patients (n= 100)</td>
<td>12.61± 1.7</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td></td>
<td>Control (n= 100)</td>
<td>1.92± .48</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>patients(n= 100)</td>
<td>6.16± 1.5</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td></td>
<td>Control(n= 100)</td>
<td>1.77± .82</td>
<td></td>
</tr>
</tbody>
</table>

P < 0.05 statically significant

comparison mean of serum antibet2-glycoprotein1( IgG ) (10.57 ± 6.5 u/ml) patients group and the control group (3.8± 2.5 U/ml). It shows that there is significant difference in the mean values P. = 0.00. and insignificant differences in mean of Bet2glycoprotin1IgM in patients group compared to control (7.1± 2.932U/mL) versus (5.68± 2.93U/mL) P .value (0.83)
Table 2: Frequency of anticardiolipin in the study population group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Numbers</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>patients (n=100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>32%</td>
</tr>
<tr>
<td>Negative</td>
<td>68</td>
<td>68%</td>
</tr>
<tr>
<td>Control (n=100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>negative</td>
<td>98</td>
<td>98%</td>
</tr>
</tbody>
</table>

Results showed that frequency of anticardiolipin among case group is 32% positive, 68% is negative, and among the control group only 2% is positive, 98% is negative. Table 2

Table 3: Frequency of Anticardiolipin (IgA, IgG, IgM) among patients group (n=100)

<table>
<thead>
<tr>
<th>Anticardiolipin u/ml</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA &amp; IgG</td>
<td>1 (1%)</td>
<td>99 (99%)</td>
<td>100 (100%)</td>
</tr>
<tr>
<td>IgG&amp;IgM</td>
<td>4 (4%)</td>
<td>96 (96%)</td>
<td>100 (100%)</td>
</tr>
<tr>
<td>IgG (alone)</td>
<td>25 (25%)</td>
<td>75 (75%)</td>
<td>100 (100%)</td>
</tr>
</tbody>
</table>
Frequency of Anticardiolipin antibodies were positive, IgG is 25% , IgM is 2%, IgG&IgA is 1%, IgG&IgM is 4%, total positive tests were 32%. And negative tests IgG is 75% IgM is 98%, IgG&IgA is 99%. IgG&IgM is 96%. total negative tests were 68% Table 3

Table 4: Comparison of anticardiolipin antibodies (IgA, IgG, IgM) levels according to the timing of miscarriage among patients group

<table>
<thead>
<tr>
<th>The anticardiolipin antibody</th>
<th>First trimester mean±SD</th>
<th>Stander trimester mean±SD</th>
<th>P- Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>4.4±1.5 U/ml</td>
<td>5.1±2.2 U/ml</td>
<td>P &lt;.016</td>
</tr>
<tr>
<td>IgG</td>
<td>11.88± 12.2 U/ml</td>
<td>14.25± 13.6 U/ml</td>
<td>p&lt;.0.41</td>
</tr>
<tr>
<td>IgM</td>
<td>5.63± 8.20 U/ml</td>
<td>7.32± 14.6 U/ml</td>
<td>P &lt;.055</td>
</tr>
</tbody>
</table>

p-values < 0.05 statistically significant
There was no significant difference between The anticardiolipin antibody (IgA, IgG, IgM) and timing of miscarriage (whether occur in the first or second trimester). ) first trimester; mean ±SD was (4.4± 1.5U/mL), (11.88± 12.2 U/mL), (5.63± 8.20 U/mL) compared with second trimester ; mean ±SD (5.1± 2.2U/mL), (14.25± 13.6 u/mL), (7.32± 14.6 U/mL) among these women with recurrent miscarriage P value (0.16), (0.41), (0.55) respectively, table 4
Figure 1 shows the relationship between the serum anticardiolipin and Bet2glycoprotin1 significant negative correlation. ($r = 0.239$, $P = 0.017$)
Discussion:
In this study we evaluated the prevalence of serum anticardilipin antibodies (IgA, IgG, IgM) and serum anti bet2-glycoporotin1 among some Sudanese women with recurrent miscarriage:
This study observed that there was significant different in the mean of serum anticardiolipin(IgA),(IgG) and IgM in the case group compared to the control group. This finding was consistent with that reported by Akhlaghi F et al[15], Bagger et al[16]. In this study there was significant increase in mean of serum beta2glycoprotin1 in patients group, compared to control group (10.57± 6.5 u/ml), (3.8 ± 2.5u/ml), P value (0.000), this agrees by, Zammiti W et al[17].

The present study revealed that there was no significant difference between anticardiolipin antibodies (ACA) and timing of miscarriage (whether occur in the first or second trimester). Similarly, Al-Hilli et al[18], Jwad et al[19], found that the presence of ACA led to same frequency of unsuccessful pregnancies in the first and second trimester. study shows the relationship between the serum anticardiolipin and Bet2glycoprotin1 significant negative correlation, this agrees by Lončar D et al[20].

In the study showed 32% of patient with recurrent miscarriage had positive anticardiolipin antibodies, compared with 2% control group. Similar findings were found in studies carried out in Assiut, Egypt in 2011[21] It was found that 85 (35.4%) out of 164 women who had recurrent spontaneous miscarriages were positive for IgG / IgM to cardiolipin[21]. Another study done by Mishra et al[22], showed that anticardiolipin antibody test was positive in 28.3% of patient with recurrent miscarriage. Velayuthaprabhu and Archunan[15] studied 155 patients with recurrent miscarriage & found that 40% of them were positive for anticardiolipin antibody[23]. A study in Jordan in 2001, found that in a group of 26 women defined as habitual aborters, 19.23% had positive ACA test results as compared with none of the control group[24]. Also found in northern Italy a raised ACA in 19% of women with miscarriage history, compared to 3% in the control group[25]. According to previous reports the frequency of aCL in recurrent pregnancy loss ranged from 11% to 42%[26,27]. In Sudan, some studies reported the prevalence of anticardiolipin antibodies (ACA). Studied done on hundred women with RM, who found 20% positive anticardiolipin antibodies(IgG) [28]. Another study in Sudan reported that 52 women with RM demonstrated a prevalence of 22.7% ACA.) [29]. The variation in the prevalence of
these antibodies in different studies may be due to the different types of fetal wastage included, different types of antiphospholipid studied & the different localities in which studies were performed and different protocols of various laboratories used to detect anticardiolipin antibodies [18].

Conclusion

From our data we can conclude that The presence of anticardiolipin antibodies (ACL), antitet2 glycoporotin1 (Aβ2GPI ) in sera of women with RM with RM(recurrent miscarriage were significantly associated with recurrent miscarriage. There was a significant correlation between levels of IgG ACL and IgG Aβ2GPI . The present study revealed that there was no significant relationship between ( IgA, IgG IgM) anticardiolipin antibodies and timing of miscarriage (whether occur in the first or second trimester). The frequency of positive anticardiolipin among case group was 32% while it was 2% among control group.

References:


` :www.euroimmun.com/.


