Prevalence of Anticardiolipin Antibodies in Pregnant Women with Recurrent Miscarriage in Al–Hilla city

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Abstract
In the present study determine the level of serum anticardiolipin (ACL) antibodies (ACL- IgM and ACL-IgG) in 30 pregnancies women who clinically diagnosed with recurrent miscarriage more than two times their aged between 21–42 years, attending to Babylon hospital of maternity and children and 15 healthy pregnant women without previous miscarriage were included as control in the study which extended from April 2013 to October 2013. Blood samples and the required information regarded with maternal age and number of previous miscarriage were collected from both patients and healthy. It was shown from the results that 4 (13.34 %) cases of pregnant women with previous miscarriage were positive in ACL-IgM and 6 (20 %) cases of pregnant women with recurrent previous miscarriage give positive for ACL-IgG, while all results give negative in healthy pregnant women. An obvious significant differences at probability level (P < 0.05) found when make comparison between ACL-IgM and ACL-IgG level in both patient and healthy.

Key word: anticardiolipin antibodies, recurrent miscarriage, risk factor

Introduction
The plasma membranes of mammalian cells are formed from phospholipids, anionic phospholipids (eg, phosphatidylserine) are found on the cytoplasm surface and neutral phospholipids (eg, phosphatidylcholine) predominate on the external surface. Membrane phospholipids participate in several important cellular functions including exchanging metabolites across membranes, transferring molecular signals and serving as a platform for the assembly of protein-lipid complexes (Bevers et al., 1999; Velayulhaprabhu and Archunan, 2005). Complexes of negatively charged (anionic) phospholipids and endogenous plasma proteins provide epitopes recognized by natural autoantibodies (Arnout and Vermyleen, 2003). A cardiolipin is a phospholipid or lipid molecule in blood, synthesized in mitochondria and it is an important component of metabolically active cells of the heart and skeletal muscles (Houtkooper and Vaz, 2008).
Plasma from normal individuals contains low concentrations of natural IgG autoantibodies of moderate affinity. Pathologic levels of autoantibodies reflect loss of tolerance and increased production of antibodies, these autoantibodies are called phospholipid or cardiolipin antibodies (Salazar et. al., 2002) they are detected by enzyme linked immunosorbent assays (ELISAs) in which the negatively charged phospholipid is coated on plastic plates as substrate, the most commonly used phospholipid substrate is cardiolipin (Castro and Gourley, 2010). Anticardiolipin antibody syndrome is usually an acquired condition, therefore it can not transmit from mother to child. The levels of anticardiolipin antibodies IgM and IgG are often high in people with abnormal blood clotting, autoimmune disease lupus (SLE) or recurrent miscarriage (Ong et. al., 2002). Recurrent miscarriage is usually defined as three or more consecutive, spontaneous miscarriages occurring in the first trimester in which many factors play a role such as maternal age, genetic, hormonal disorders, uterine factors, the infections, environmental and immunologic factors (Clarisa et. al., 2005). A successful birth may or may not follow; about one half of recurrent miscarriages are unexplained, antiphospholipid syndrome is one of the known causes of first- and second-trimester recurrent miscarriage (Ruiz-Irastorza et. al., 2010). Antiphospholipid syndrome (APS) is defined as the presence of anticardiolipin antibodies or lupus anticoagulant antibodies in association with three or more consecutive fetal losses before 10 weeks of gestation (Choudhury and Knapp, 2001), one or more unexplained intrauterine deaths after 10 weeks of gestation, one or more premature births before 34 weeks of gestation due to severe preeclampsia or impaired fetal growth. Many studies have shown the correlation between these autoantibodies and enhanced incidence of abortion in pregnant women rate from 0.2% to 2% is similar to the frequency in the general population. Antiphospholipid antibodies are present in 15 percent of women with recurrent miscarriage (Rai et. al., 1995; Heilmann et. al., 2003; Kareem, 2012; Zakarea et. al., 2013). In Iraq there are number of studies to evaluate the relationships between the incidences of anticardiolipin with recurrent miscarriage (Jwad et. al., 2006; Risan, 2011). So, the aim of study was to determine the prevalence of anticardiolipin antibody (ACA) in women with recurrent miscarriage in Al-Hilla city.

**Material and Method**

**1- Patients and healthy**

The present study was conducted in Babylon teaching hospital for maternity and children during April 2013 to October 2013 on 30 pregnant women who had suffered from two or more time of recurrent miscarriage and 15 healthy pregnant women as control. The patients age range between 21- 42 years. The required information regarded in maternal age and number of previously birth losses was collected by personal interview from the pregnant women.

**2- Specimens**

Three ml of blood sample was collected from both groups of pregnant women, the whole blood samples were centrifuged for 5 minute at 3000 rpm. The sera kept frozen at – 20 C° until performed the test, all specimens will treated and reading in measuring of ACL- IgG and ACL- IgM by ELISA method in Chorus Trio (DISSE Diagnostica Senese – Monteriggioni- Italy) system at Merjan teaching hospital and compare the readings result with accompanied reference values according to instruction of manufacturer’s company as follow:
Normal: IgG < 12 GPLU/mL, IgM < 12 MPLU/mL
Doubtful: IgG = 12 – 18 GPL U/mL, IgM = 12 – 18 MPL U/mL
Positive: IgG > 18 GPL U/mL, IgM > 18 MPL U/mL

MPL refers to IgM Phospholipid Units. One MPL unit is 1 microgram of IgM antibody. GPL refers to IgG Phospholipid Units. One GPL unit is 1 microgram of IgG antibody.

3- Statistical analysis:
Data were analyzed using SPSS (statistical package for social science). Mean, Standard deviation and t- test (P- value < 0.05) were used as statistical parameters in this work.

Results
Table (1) showed the occurrence of ACL- IgM in both studied groups, there are 4 (13.34%) cases of pregnant women with recurrent miscarriage give positive to ACL-IgM while 26( 86.66 %) of them give negative result as compared with healthy pregnant women give negative.

Table 1: Distribution of pregnant with recurrent miscarriage and healthy pregnant according to anticardiolipin IgM

<table>
<thead>
<tr>
<th>Anticardiolipin Ab IgM</th>
<th>Pregnant with recurrent miscarriage %</th>
<th>Healthy pregnant %</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4 (13.34)</td>
<td>---</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>26 (86.66)</td>
<td>15 (100)</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>15</td>
<td>45</td>
</tr>
</tbody>
</table>

Table (2) expressed that 6 (20 %) for pregnant women with recurrent miscarriage give positive result in testing sera to ACL-IgG while 24 (80 %) of them give negative result compared with healthy women.

Table 2: Distribution of pregnant with recurrent miscarriage and healthy pregnant according to anticardiolipin IgG

<table>
<thead>
<tr>
<th>Anticardiolipin Ab IgG</th>
<th>Pregnant with recurrent miscarriage %</th>
<th>Healthy pregnant %</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6 (20)</td>
<td>---</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>24 (80)</td>
<td>15 (100)</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>15</td>
<td>45</td>
</tr>
</tbody>
</table>

Table (3) shows there is a significant differences (P < 0.05) when compared the level of ACL- IgM and ACL-IgG between the pregnant women with recurrent miscarriage and healthy pregnant women.
Table 3: level of anticardiolipin antibodies IgM, IgG (PLU/mL) in sera of pregnant with recurrent miscarriage and healthy pregnant women

<table>
<thead>
<tr>
<th></th>
<th>ACL- IgM</th>
<th>ACL- IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant with recurrent miscarriage</td>
<td>M</td>
<td>19.844</td>
</tr>
<tr>
<td>N. 30</td>
<td>SD ±</td>
<td>26.977</td>
</tr>
<tr>
<td>Healthy pregnant</td>
<td>M</td>
<td>5.5000</td>
</tr>
<tr>
<td>N. 15</td>
<td>SD ±</td>
<td>2.0410</td>
</tr>
<tr>
<td>Significance</td>
<td>Significant</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

M= mean  SD= standard deviation

Discussion
Antibodies against cardiolipin belong to the group of antiphospholipid antibodies (APL) a heterogeneous group of autoantibodies directed against negatively charged phospholipids a component of biological membrane (Reddel and Krilis, 1999). The anticardiolipin ACL assay serves to assist in diagnosis of APL that is important for treatment and predication of thrombosis and recurrent miscarriage (Branch et al., 2000; Cowchock, 1996).

The current study detected high level of serum anticardiolipin ACL-IgM 4 (13.34 %) among recurrent miscarriage women with significant difference as compared with healthy women tables (1, 3) and increasing in level of ACL- IgG 6 (20 %) with significant difference as compared with healthy women tables (2, 3). The persistence presence in plasma of medium to high level of IgG and IgM class anticardiolipin antibodies and or Lupus anticoagulant is associated with both recurrent pregnancy loss and venous thrombosis (Derkson, 2001). Many studies agreed with our results that are found a significant association between repeated miscarriage and presence of high level of ACL-Abs. for example in Iraq the worker Jawad et. al. 2006 found (17.6 %) the rate of ACL-Abs. in pregnant women with fetal losses, another study by Risan, 2011 found high titer of ACL-IgG is of clinical significance in identifying women at a risk of pregnancy loss then ACL-IgM antibodies. A study from Jordan in 2001, found that in a group of 26 women defined as habitual abortion, 19.23 % had positive ACL- Abs. as compared with control group (Daboubi, 2001). Similar findings were found in study carried out in Brazil, high level of ACL- IgM (41.1%) and (17.6%) for ACL- IgG (Spegiorin et. al., 2010). The present study is consistent with the other studies like (Zakarea et. al., 2013; Rai et. al., 1995; Al-Abri et al., 2000). In different studies there is high a variation in rate of ACL-Abs. may be due to many causes of repeated abortion in
women and the different methods that employed to determinate antibodies so, these data indicate the necessity of systemic investigation of these antibodies in pregnant women at risk of miscarriage.

References


Jawad, IM.; Mahdi, NK. and Flafil, MS. (2006). Anticardiolipin antibody in women with recurrent spontaneous miscarriage. 27(9).


