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The Prevalence of Positive Serum Anticardiolipin Antibodies and Asymptomatic Bacteriuria in Women with Recurrent Abortions

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Abstract

Objective: The asymptomatic colonization of the urinary tract in pregnant women may result in severe medical and obstetric complications. The aim of this study was to study the prevalence of asymptomatic bacteriuria in cases of elevated levels of the anticardiolipin antibody in women who experience spontaneous abortions.

Materials and Methods: A total of 12 women were enrolled in this case control study, including 60 patients with a history of three or more abortions and 60 healthy pregnant women. All participants were screened for ACL (IgG) and with a urine culture.

Results: Overall, 19 (31.7%) patients and seven (11.7%) healthy pregnant women were positive for ACL. The mean concentrations were 67.1±27.2 IU/mL in the patients and 17.41±6.12 IU/mL in the healthy controls (p≤0.05). In the 60 patients, only 17 (28.3%) had significant bacteriuria, whereas 5 (8.3%) women in the control group had significant bacteriuria. The statistical analysis revealed a highly significant difference. Of the 19 patients with a positive elevation of ACL, 11 (57.9%) had significant bacteriuria, and eight (42.1%) had non-significant bacteriuria. Six patients had ACL-negative results associated with significant bacteriuria. The statistical analysis revealed a highly significant difference.

Conclusion: A high serum anticardiolipin level was prevalent in women who experienced recurrent abortions associated with asymptomatic bacteriuria.

Key Words: Abortion, Anticardiolipin, Asymptomatic bacteriuria

Introduction

There are different causes of abortion, and many factors, such as hormonal, genetic, and anatomic features, systemic hypertension, infections, diabetes, and hyperthyroidism, are well known to cause this issue [1]. Immunological factors are also involved in these cases [2] and are one of the most remarkable causes of abortion [3]. The elevation of the antiphospholipid antibody plasma level (aPL) has been reported to be one of these factors. These antibodies include

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different groups of autoantibodies that form against phospholipoproteins that have negative charges in the body. These antibodies include antiphospholipid (aPL), lupus anticoagulant (LA), anti-beta2-glycoprotein 1 (aB2-GP-1), antiphosphoserine (aPS), anti-phosphatidylycerol (aPG), anti-phosphatidic acid and anti-phosphatidyl inositol [4-6]. Among these antibodies, antiphospholipid and lupus anticoagulant are known to have more critical and significant roles in spontaneous abortion [7].

There are three major types of urinary tract infections in pregnancy: asymptomatic bacteriuria, acute cystitis and acute pyelonephritis. Asymptomatic bacteriuria is defined as a finding of more than 10^5 colony forming units per milliliter of urine in a clinically asymptomatic person. The asymptomatic colonization of the urinary tract in pregnant women may result in severe medical and obstetric complications [8, 9]. The aim of this study was to study the prevalence of asymptomatic bacteriuria in cases of elevated levels of antiphospholipid antibodies in women who experienced spontaneous abortions.

**Materials and Methods**

**Study protocol and Sampling**

This study (January 2010-June 2012) was approved by the Ethics Committee of Hawler Medical University, Erbil, Iraq. Informed consent was obtained from all participants. A total of 12 women (60 patients who had abortions and 60 healthy controls) were enrolled in this case control study. The strictly selected patient group had a history of three or more consecutive pregnancies. Informed consent was obtained from all participants. A total of 12 women (60 patients who had abortions and 60 healthy controls) were enrolled in this case control study. The strictly selected patient group had a history of three or more consecutive pregnancies. The exclusion criteria were the following: a history of uterine anomalies; diabetes mellitus; thyroid disease; aspirin, heparin, antibiotic or corticosteroid intake; embryo anomalies; chronic systemic disease including lupus, autoimmune diseases, hypothyroidism, asthma, and cardiopulmonary diseases and a previous history of a symptomatic urinary tract infection. In addition, investigations for the hormonal assays and infections (CMV, rubella, toxoplasmosis) were negative. The controls included women with a previous normal pregnancy who were para 3 and gravida 4. The diagnosis was approved according to clinical criteria and the partial thromboplastin time [10, 11].

**Analytical methods**

A 5 ml aliquot of blood was taken from each patient. The separated serum was stored frozen at -30°C until analyzed for ACL. The detection of ACL IgG was conducted using the ELISA technique as described by the manufacturer of the kit (ACL ELISA Kit, Orgentec Diagnostika GmbH, Mainz, Germany). According to the manufacturer’s instructions, the result was considered negative if <10 GPL IU/mL and positive if >10 GPL IU/mL. Each participant was asked to provide a clean-catch midstream early morning urine sample. The patients had been instructed to clean the vulva thrice from front to back each time with a separate piece of sterile gauze soaked in saline and to then use a wide-mouth 50 ml sterile bottle to collect the urine sample starting approximately two seconds after the beginning of the flow [8, 9]. The culture was performed on nutrient agar and in differential Mckonkey agar. The approximate number of bacteria per milliliter of uncentrifuged urine was estimated using a sterile, specially calibrated urine loop that can hold 1/500 mL, i.e., 0.002 mL, of urine. The urine from the urine loop was inoculated on a sterile culture and incubated aerobically at 35-37°C for 24-48 hours. The number of incubated colonies was counted using a counting chamber and then multiplied by a factor of 500 to estimate the number of bacterial colonies/mL, i.e., CFU/mL. A count of more than 10^4/mL of urine was considered to be significant. Counts between 10^4-10^5/mL of urine were considered doubtful, and the sample was re-examined. A count of less than 10^5/mL of urine was considered to be not significant. Each significant isolate was identified by colony morphology, gram staining and biochemical reaction according to standard procedures [12, 13].

**Statistical analysis**

The data were expressed as the mean±standard deviation or as percentages and ranges as appropriate. Comparisons were made using the Student t test and Chi x^2 test using standard equations. The results were reported with p≤0.05 as the accepted level of significance.

**Results**

Table 1 illustrates the distribution of ACL positive results in addition to the mean±S.D and range of the serum ACL levels in both the patient and control groups. In the patient group, 19 (31.7%) women had a positive elevation of the ACL levels with a mean±S.D and range of 67.1±27.2 IU/mL and 23.5 - 119.5 IU/mL, respectively. In the control group, only 7 (11.7%) pregnant women had a positive ACL with a mean±S.D and range of 17.6±6.12 IU/mL and 10.5–25 IU/mL, respectively. The difference in the means between both groups was highly significant by the Student t test (p<0.05).

Table 2 shows the distribution of results of the urine culture in both the patient and control groups. Of the 60 patients, only 17 (28.3%) had significant bacteriuria, whereas in the control group, 5 (8.3%) women had significant bacteriuria. The statistical analysis revealed a highly significant difference.

Table 3 illustrates the distribution of the results of the urine cultures in both the ACL-positive and -negative patients. In the 19 patients with a positive elevation of ACL, eleven (57.9%) had significant bacteriuria, and eight (42.1%) had
non-significant bacteriuria. Six patients had ACL negative results associated with significant bacteriuria. The statistical analysis revealed a highly significant difference.

**Discussion**

This study showed that 31.7% of the women who experienced recurrent abortions of unknown causes attending the maternity hospital in Erbil City had elevated ACL levels. In addition, 57.9% of the patients who had elevated ACL levels also had significant asymptomatic bacteriuria. Since 1983, many infections have been found to be associated with aPL, although a pathogenic role for these antibodies has not been obvious except in a few isolated cases. Recently, many infections have been emphasized and reported on several occasions to not only potentially trigger the production of these antibodies but also be accompanied by the clinical manifestation of APL itself [14].

**Table 1. Mean and range of the ACL concentration in the patients and controls**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Mean±S.D (IU/mL)</th>
<th>Range (IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>19</td>
<td>67.1±27.2</td>
<td>23.5 - 119.5</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>17.6±6.12</td>
<td>10.5 - 25</td>
</tr>
<tr>
<td>t = 3.58</td>
<td>df = 24</td>
<td>Highly significant (p≤ 0.05)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Distribution of significant and non-significant bacteriuria in both the patient and control groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Significant bacteriuria</th>
<th>Non-significant bacteriuria</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>17</td>
<td>43</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>98</td>
<td>120</td>
</tr>
<tr>
<td>Chi-square value = 7.07</td>
<td>df =1 Highly significant (p≤ 0.05)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Distribution of significant and non-significant bacteriuria in both ACL-positive and -negative patients**

<table>
<thead>
<tr>
<th>Groups</th>
<th>ACL Positive</th>
<th>ACL Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant bacteriuria</td>
<td>11</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Non-significant bacteriuria</td>
<td>8</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>41</td>
<td>60</td>
</tr>
<tr>
<td>Chi-square value = 10.98</td>
<td>degrees of freedom =1 Highly significant (p≤0.05)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

phospholipid antibodies as a heterogeneous group of circulating immunoglobulins arising in a wide range of infections and autoimmune diseases. McNeil et al. [17] and Asherson et al. [18] reported the presence of antiphospholipid antibodies in infectious diseases such as HIV, tuberculosis, measles, chickenpox, hepatitis, parvovirus and rubella. In a study on 100 patients complaining of antiphospholipid syndrome, Cervera R et al. [14] found that a urinary tract infection was the associated infection in 10% of these patients. Asherson et al. [18] reported that 35% of catastrophic APS cases were preceded by an infection, with urinary tract infections reported to occur in 6% of the cases. A molecular mimicry mechanism between the pathogen and β2GP1 has been suggested to be the potential cause of APS based on the following: 1- the correlation between APS clinical manifestations and infectious agents in humans and 2- the strong homology between β2GP1-related peptides (target epitopes for anti-B2GP1 antibodies) and different common pathogens in the protein databases [19]. Considerable reported evidence has suggested that β2GP1 is the main antigenic target for aPL. An *S. aureus* protein (Sbi) that binds β2GP1 had been reported to serve as a target molecule for IgG binding [14]. In addition to its presence in the plasma, β2GP1 is expressed on the surface membranes of different cell types involved in the pathogenesis of the syndrome, including endothelial cells, monocytes and trophoblasts [20]. Anti-B2GP1 antibodies may recognize and cluster the molecules bound to its own endothelial cell membrane receptors, eventually inducing the signaling events that lead to the induction of the proinflammatory and procoagulant phenotype [21]. Raschi E et al. [22] showed that anti-β2GP1 antibodies induce an endothelial signaling cascade comparable with that activated by lipopolysaccharides (LPS) through the involvement of the toll-like receptor, TLR-4. Shoenfeld Y et al. [19] speculated that β2GP1 alone or complexed with its own endothelial cell membrane receptors might interact with TLRs and that anti-β2GP1 antibodies that recognize the molecule might crosslink it with TLRs, eventually triggering the signaling cascades.

Evidence suggests that the putative β2GP1 phospholipid binding site might be involved in the binding to anionic endothelial cell structures, such as heparin sulfate, and to annexin A2, the receptor for plasminogen/tissue plasminogen activator [23].

Annexin A2 has been suggested to require TLR-4 as a co-receptor to signal because annexin A2 binds β2GP1 with high affinity but does not display any transmembrane protein [23, 24].

TLR-4 is a key component of the innate immune response and can recognize specific microbial products, including LPS. Being transmembrane proteins, all TLR family members behave as efficient receptors, able to drive a prompt inflammatory response after their interaction with specific ligands [24].
A two-hit hypothesis has been suggested to explain the common clinical observation that aPL might be persistently present even though thrombotic events occur only occasionally: aPL (first hit) increases the thrombophilic risk, and clotting occurs in the presence of another thrombophilic condition [25].

Fischetti et al. [26] hypothesized that the involvement of TLRs by microbial structures combined with that mediated by anti-β2GP1 antibodies might synergistically contribute to the second hit that triggers the clotting event.

Such a possibility is in line with a recent in vivo experimental model. Human anti-β2GP1 IgG infused into a naïve rat does not significantly affect the mesenteric microcirculation, but the same IgG fractions trigger clotting if a primary proinflammatory factor, such as LPS, is present. These findings suggest a role for infectious agents as a second hit and the involvement of receptors of innate immunity at the same time [20].

In conclusion, based on this study, searching for and detecting asymptomatic bacteriuria may be of benefit for preventing spontaneous abortions related to high anticardiolipin antibody levels.

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**References**