The Role of Anti-Phospholipid Auto Antibodies Syndrome in Cerebrovascular Diseases

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Abstract:

Objectives: The aims of the study are to determine the role of anti-phospholipid autoantibodies (APLAs) among patients with stroke and/or transient ischaemic attacks (TIAs), to identify the types and effective isotype of some important APLAs which are lupus anticoagulant (LA), anticardiolipin (aCL), anti-β2 glycoprotein I dependent (αβ2-GPI), and anti-phosphatidyl serine (aPS). Also, to find out any concomitant effect of non-APLA parameters not specific to antiphospholipid syndrome (APS).

Subjects and Methods: This study was carried out on 50 patients attending mainly the Teaching and General Hospitals in Mosul, Duhok, and Erbil Cities, Iraq during the period between 1st March 2004 and 1st March 2005. The studied cases were under 50 years of age, and had no recognizable risk factors. The activated partial thromboplastin time (APTT) was used for LA estimation and ELISA assay was used to test for IgG & IgM isotypes of aCL, αβ2-GPI, and aPS. The ANA, RF, VDRL, CRP, and C4 complement were tested by different immunoserological methods. The thrombocytopenic (TP) status was diagnosed by estimation of platelets counts.

Results: The strokes and/or TIAs related to APS were diagnosed in 22/50 (44%) of patients and a significant correlation was reported among patients with IgG plus IgM aCL (p < 0.05), IgG αβ2-GPI (p < 0.05), and IgG aPS (p < 0.05). In APLAs positive patients, raised CRP concentrations were reported in 36.4%, TP status in 22.7%, ANA in 22.7%, RF positive in 13.6%, and low C4 levels in 13.6%. Finally, the FP-VDRL test was found in 50% of APLAs positive cases.

Conclusions: The aCL, αβ2-GP, and aPS antibodies were shown to play a significant role in the development of stroke and/or TIAs among the studied cases.

Key words: Anti-phospholipid antibodies, stroke and/or TIAs

Introduction:

The antiphospholipid syndrome (APS) is an autoimmune disease characterized by existence of antiphospholipid antibodies (APLAs) and at least one clinical manifestation, the most common being venous or arterial thrombosis and recurrent fetal loss(1-2). The APLAs are heterogenous group of autoantibodies that are detected by immunoassays and functional coagulation tests. The syndrome is now widely accepted as a systemic autoimmune disease initially identified in the setting of systemic lupus erythematosus (SLE), APS was then found in patients without any other full blown disease, allowing the definition of primary and secondary APS(3,4,5).

Lupus anticoagulant (LA) and anticardiolipin (aCL) antibodies are the most important clinical types of APLAs which are associated with clinical APS with varying severity(6,7). Another antibody system called anti-β2-GPI (αβ2-GPI) has been identified in patients with APS and found to play an important role in pathogenesis of this syndrome(8). For LA, the complex of phospholipid-bound prothrombin is binding site(9,10), while in case of aCL antibodies the β2-GPI is the main protein cofactor(11). The type of antibody in aCL are often of IgG isotype, usually are persistence and associated with an LA phenomenon in about 60% of cases(11). Other autoantibodies against negatively-charged phospholipids rather than aCL and LA were also mentioned such as Phosphatidyl serine (aPS)(13,14).

There are a substantial number of gaps in the knowledge about the association between stroke and APS(15). The APS should be suspected in young patients with transient ischemia attacks (TIA) or stroke, particularly in the absence of the usual risk factors for cerebrovascular disease(16). Therefore, the aims of this study is to find out such an association among patients with APS.

Materials and Methods:

This study was carried out on 50 patients suffering from stroke and/or TIAs attending mainly the Teaching and General Hospitals in Ninevah, Duhok, and Erbil Governorates, Iraq during the period between 1st March 2004 and 1st March 2005. The cases were selected to be less than 50 years of age and
having no recognizable risk factors including hypertension, diabetes mellitus, dyslipidemia, smoking, and lastly not overweight. The cases should not be previously diagnosed as systemic lupus erythematosus.

The age range of the studied cases was 25-48 years (mean ± SD, 36.2 ± 6.7). The stroke attacks were reported in 36/50 (72%) and TIA’s in 14/50 (28%). Among the stroke cases, single episode was detected in 26/36 (72.2%) and recurrent episodes in 10/36 (27.8%). Ischaemic strokes were defined by clinical criteria as focal neurological deficits of sudden onset lasting ≥ 24 hours, most with confirmatory Computerized Tomography (CT) scanning or Magnetic Resonance Imaging (MRI). TIA were similarly defined, with symptoms lasting < 24 hours and negative brain imaging. These cases were clinically diagnosed by neurologists and confirmed by image techniques.

All the laboratory kits and reagents used throughout this study were purchased from international suppliers and companies. The Coagulation assay kit (Cephalite kit Biokit, S.A, Barcelona-Spain) was used for the estimation of APTT. The Enzyme-immunoassay kits (Orgentik Diagnostika Mainz/ Germany) were used for the detection of aCL, αβ2-GP I, and aPS of both IgG and IgM isotypes. Different immunosorbergological kits were also used for VDRL test (Biomaghreb Tunisia). Latex agglutination tests for the detection of rheumatoid factor and anti-nuclear antibody (Labbik, Canovelles-Barcellona-Spain), and C-reactive protein (Biokit, Barcelona, Spain), and C4 complement component estimation by single radial immunodiffusion test (KENT LAB. INC. Washington, USA).

The APTT results greater than the upper limit of normal (30-40 seconds) were considered to be abnormal and confirmatory test was carried out by mixing 1:1 of the tested plasma sample with normal pooled plasma (consisted of plasma from at least 10 normal controls) in a tube. If an LA was present, the initially prolonged clotting time would not be corrected. The result for the APTT was considered to be positive only if both screening and confirmatory tests were abnormal. An indirect solid phase enzyme immunoassay (ELISA) technique was used for the quantitative measurement of IgG and IgM class antiphospholipid autoantibodies. The following ranges were adopted according to the manufacturer’s instructions:

The enrolled individuals from both groups studied were undergone a complete blood picture and platelet counts as a routine laboratory test and all cases with thrombocytopenia were registered. The cases were subdivided according to platelets count into those with mild thrombocytopenia (101-140 X 10^9/L), moderate thrombocytopenia (51-100 X 10^9/L), and severe thrombocytopenia (0-50 X 10^9/L).

A significant relative risk factor was considered when the OR was greater than 1. The proportions were compared using the Chi-square test with df1 or df (r-1) (c-1) wherever indicated. The 95% CI of proportions were calculated according to the binomial distribution. The significant level was set at p < 0.05.

Results:

The frequency of APLAs

The detected frequencies of the different APLAs in patients with stroke and / or TIA attacks, and the controls are shown in Table 1. The LA activity was detected in 8/50 (16%) cases and 1/30 (3.3%) of the control, with non-significant difference. The aCL IgG isotype (concentration > 10 units/ml) was seen in 13/50 (26%) cases and among 4/30 (13.3%) of the controls, but with non-significant value. The frequency rate for cases with concentration of IgG aCL > 30 units was seen in 7/50 (14%) patients, with significant difference. The IgM aCL frequency rate (concentration > 7 units/ml) was detected in 4/50 (8%) patients, with non-significant value, while none of the controls was positive. Two of the 4 cases were also positive for IgG isotype. Therefore, the frequency rate for detection of both IgG with IgM aCL was 15/50 (30%), with significant value.
The frequency rate of IgG a/β2-GPI detection (concentration > 8 units/ml) was seen in 11/50 (22%) cases and 2/30 (6.7%) among the controls, with significant difference. The frequency rate for IgM a/β2-GPI (concentration > 8 units/ml) was 4/50 (8%), with non-significant difference, while none of the controls was positive. Two of the 4 cases were also positive for IgG isotype. Therefore, the frequency rate for detection of both IgG with IgM a/β2-GPI was 13/50 (26%), with significant difference.

The IgG aPS (concentration > 10 units/ml) was seen in 9/ 50 (18%) cases and in only 1/30 (3.3%) of the controls, with significant difference. The frequency of IgM aPS (concentration ≥ 10 units/ml) was detected in 2/50 (4%) patients and none of the controls were positive, but with non-significant difference. These 2 cases were also positive for IgG (i.e., the frequency for detection of both IgM and IgG aPS was the same as for IgG isotype). Therefore, 22/50 (44%) of stroke and/or TIAs patients and 6/30 (20%) of the controls were positive for one or more of the APLAs, with significant difference.

The presence of other clinical events related to APS was seen in 4/22 (18.2%) among the APLAs positive cases. The history of MI was observed in 3 (2 with recurrent stroke attacks and one with single attack) cases and one with repeated abortions with single stroke attack.

**The frequency of non-APLA parameters**

The frequencies of the six non-APLA parameter tests performed for the patients and the controls are shown in **Table 2**. Among patients, the FP-VDRL was seen in 16/50 (32%) and 3/30 (10%) of the controls, with significant difference (P < 0.05). The FP-VDRL test was detected in 11/22 (50%) among stroke and/or TIAs APLAs positive patients and in 5/28 (17.8%) in negative cases, with significant difference (p < 0.025). Ten of the 11 cases with FP-VDRL had also aCL, besides being positive for other parameters.

The high CRP (≥ 12mg/l) levels was detected in 10/50 (20%) cases and 2/30 (6.7%) of the controls, but without significant difference. The frequency of high CRP levels was detected in 8/22 (36.4%) positive patients and 2/28 (7.1%) in negative cases, with significant difference (p < 0.025). Three of the 8 patients with high CRP levels had concomitant history of MI or repeated abortions.

The low C4 levels was seen in 4/50 (8%) cases and 2/30 (6.7%) of the controls, without significant difference. Low C4 levels were seen in 3/22 (13.6%) among stroke and/or TIAs APLAs patients and 1/28 (3.6%) in negative cases, with non-significant difference. The 3 cases were positive for aCL, while...
aPS antibodies with αβ2-GPI was seen in 2 cases. History of MI was reported in only one case.

The ANA positive test was seen in 7/50 (14%) patients and 3/30 (10%) of the controls, with non-significant difference. Positive ANA test was detected in 5/22 (22.7%) among APLAs positive patients and 2/28 (7.1%) in negative patients, with non-significant differences. Also, the RF positive test was detected in 5/50 (10%) cases and 2/30 (6.7%) of the controls, without significant difference.

The TP status was diagnosed in 8/50 (16%) patients and 2/30 (6.7%) of the controls, with non-significant difference. The TP status was diagnosed in 5/22 (22.7%) APLAs patients and in 3/28 (10.7%) negative patients, with non-significant difference. The aCL antibody was positive in all cases, αβ2-GPI and aPS in four cases, and LA in 3 cases. Two of the patients had accompanying history of MI.

Among the stroke patients with single attack, the APLAs was detected in 11/26 (42.3%), while these antibodies were detected in 5/10 (50%) of the patients with recurrent attacks. In cases with TIAs, the APLAs were seen in 6/14 (42.8%). In cases with MI, the APLAs were seen in 5/10 (50%) cases and 2/30 (6.7%) of the controls, with non-significant difference. The aCL antibody was positive in all cases, αβ2-GPI and aPS in four cases, and LA in 3 cases. Two of the patients had accompanying history of MI.

The detection of cases with combined profiles of LA plus αβ2-GPI and aCL plus αβ2-GPI among APS stroke and/or TIAs patients indicates that αβ2-GPI antigen is the main play maker of APS in these cases(46,47,48). However, the IgG αβ2-GPI is an independent risk factor for stroke and/or TIAs events related to APS. In the current study, 2 cases of stroke attacks were found to harbor αβ2-GPI as the sole marker with absence of other APLAs. The term “antiphospholipid cofactor syndrome” has been used to define such clinical cases(44,45).

The detection of cases with combined profiles of LA plus αβ2-GPI and aCL plus αβ2-GPI among APS stroke and/or TIAs patients demonstrates the presence of APLAs (LA, aCL, or both) in 41% of patients with ischaemic stroke (13). In the present study one or more of the four specific APLAs tested were detected among 22/50 (44%) of stroke and/or TIAs selected patients.

The present study showed that LA activity carry no significant risk for stroke and/or TIAs development related to APS. However, the detection of LA and aCL antibodies (30%) and LA and αβ2-GPI antibodies (26%) among positive patients enhances the thrombogenic activity of LA. The association was significant for IgG plus IgM aCL. Several studies have suggested that aCL are not significant risk factors for these events(30,31,32), while Brey et al(33,34) reported that aCL antibodies were independent risk factors for stroke.

This study showed a significant association between stroke and/or TIAs cases and the detection of IgG αβ2-GPI as clinical events of APS (p < 0.05). Recently it has been shown that αβ2-GPI antibody may be more specific than aCL in predicting thrombosis and other clinical events related to APS(41,42). In the current study, 2 cases of stroke attacks were found to harbor αβ2-GPI as the sole marker with absence of other APLAs. The term “antiphospholipid cofactor syndrome” has been used to define such clinical cases(44,45).

The role of APLAs as novel risk factors for ischaemic stroke and TIAs remains a matter of debate(33,34). Several prospective studies have suggested that these autoantibodies to carry no significant risk factors(30,31,32), while others revealed a significant association(35,36,37). Recently, the APASS Investigators have demonstrated the presence of APLAs (LA, aCL, or both) in 41% of patients with ischaemic stroke (13). In the present study one or more of the four specific APLAs tested were detected among 22/50 (44%) of stroke and/or TIAs selected patients.

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The detection of cases with combined profiles of LA plus αβ2-GPI and aCL plus αβ2-GPI among APS stroke and/or TIAs patients indicates that αβ2-GPI antigen is the main play maker of APS in these cases(46,47,48). However, the IgG αβ2-GPI is an independent risk factor and plays a more enhanced significant role in the recurrent stroke events than in cases with single stroke attacks.

The association was significant with high relative risk factors between the frequency rates of aPS IgG with stroke and/or TIAs (p < 0.05). Tuhrim et al(49) reported a similar association. Another study by Toschi et al(50) of stroke and/or TIAs patients, but with unselected patients demonstrated a positive correlation. In the present study, 2 of the patients were aPS positive alone without other APLAs. Thus, the magnitude of the stroke risk associated with APLAs in APS may be underestimated if aPS testing is not considered.

The detected frequency rates of IgM isotype of the different studied APLAs showed no significant relative risk factors with stroke and/or TIAs events of APS. In the majority of APS cases, the pathogenic autoantibodies were rarely of IgM class(51,52,53). Therefore, APLAs IgM isotypes testing among

### Neurological findings and degree of disability

The findings of the brain CTS and MRI studies among the 22 positive patients are shown in Table 3. Normal findings were seen in 4/22 (18.2%), localized ischaemic infarcted areas in 15 (68.2%), and multiple infarcted areas in 3 (13.6%) of the positive cases. Left sided brain involvement was detected in 11/22 (50%) and right sided in 7/22 (31.8%) of APLAs positive cases, while none showed imaging evidence of hemorrhage. The disability was formulated into 4 degrees, normal disability was observed in 6/22 (27.3%), slight disability in 7/22 (31.8%), moderate in 7/22 (31.8%), and severe in 2/22 (9.1%) of patients.

#### Table 3: The neuroradiological (CTS / MRI) findings of the APLAs positive patients with stroke and/or TIA

<table>
<thead>
<tr>
<th>Neuroradiological findings CTS / MRI</th>
<th>No. 22*</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4</td>
<td>18.2</td>
</tr>
<tr>
<td>Localized infarction</td>
<td>15</td>
<td>68.2</td>
</tr>
<tr>
<td>Multiple infarction</td>
<td>3</td>
<td>13.6</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 11 cases with left sided positive brain neuroimaging findings and 7 with right sided findings; CTS = Computerized tomography scanning; MRI = Magnetic resonance imaging
stroke and/or TIAs patients have a limited value in clinical practice among patients with events related to APS.

Among the stroke patients with single attack the APS was diagnosed in 42.3% and in 50% of patients with recurrent attacks and in 42.8% of cases with TIAs. The existence of other clinical manifestations of APS such as MI and repeated abortions in the positive cases (18.2%) may point out to the heterogeneity of this syndrome. Different combination profiles of APLAs and other parameters were detected in the studied cases. Therefore, it is suggested to perform LA test in combination with other APLAs such as aCL, aβ2-GPI, and aPS to increase the frequency rate of APLAs positive stroke and/or TIAs event of APS.

The majority of FP-VDRL APLAs positive cases were also positive for aCL. This may point out to the enhanced cross reaction with aCL in inducing FP-VDRL positive tests among stroke patients. The association was lacking between the diagnosed cases of TP status and APLAs positive cases. The majority of cases with TP were with recurrent stroke attacks had different APLAs and non-APLA parameters which could indicate an ongoing autoimmune process leading to the induction of different autoantibodies to platelets. Form these findings its evident that TP status can develop as a consequence to the autoimmune process characterized this syndrome and such cases were associated mainly with recurrent stroke attacks.

The detected frequency rates of low C4 levels among the APLAs positive cases related to APS was not significant, but low concentrations were estimated in recurrent stroke cases with active disease forms with accompanying history of recurrent stroke and/or TIAs, and with neuroimaging finding of multifarcted areas. Moreover, significant levels of CRP, RF, and states of TP were recorded among APLAs positive stroke attacks. The concentration of high CRP levels (45 mg/L) among the APLAs positive cases with low C4 titers. The accumulation of these markers in such cases highlights the possible existence of inflammatory and complements activation.

The correlation between the detected frequency rate (36.4%) and the concentration of high CRP levels (45 mg/L) among the APLAs positive cases related to APS was significant. Plasma CRP is an independent novel risk factor for stroke and a marker for inflammation. Moreover, recent study has suggested that raised CRP levels is associated with high aCL rates in APS and both have similar mechanisms by which they promote vascular thrombosis. Therefore, this marker plays a significant role in the pathogenesis of stroke and/or TIAs.

The existence of ANA and RF positive cases and the detection of low C4 titers in some of the studied patients may by itself represent active disease forms and profound direct etiologic relevance of these markers in the pathogenesis of this syndrome. It may also point out to cases which may evolve into SAPS such as SLE state (with ANA and low C4 titers) or other rheumatic disease (with RF). Moreover, it could be a bystander finding or epiphenomenon, a sign of other underlying disease(s) per se, since the rates of these non-APLAs parameters in APLAs positive patients don't differ from that in patients with negative antibody tests and the controls.

All cases with recurrent stroke attacks were found to be with positive imaging findings. In cases with single stroke attacks and those with TIAs, 62.5% and 33.3% respectively were found to be with localized infarction areas. These radiological findings showed more predilection of the left sided brain involvement than the right side. The stroke suffered by patients with APLAs was found to be with slight to moderate severity (65%), while severe disability was reported in 9.1%. Therefore, these findings indicates that stroke outcome in these cases was not uniformly poor and was often good. Similar results were reported by others.

Recently, elevated serum concentrations of aCL antibodies independently of other cardiovascular risk factors were found to significantly predict the risk of future ischaemic stroke and TIA in women but not in men. In essence, the presence of APLAs has been associated with vascular occlusive events in patients with ischemic stroke, but the role of these antibodies in predicting ischemic events, particularly recurrent stroke is controversial. However, another study was found that the presence of either LA or aCL antibodies among patients with ischemic stroke not to predict the increased risk for subsequent vascular occlusive events.

In conclusion, the study demonstrate an association between aCL IgG with IgM, aβ2-GPI IgG, and aPS IgG and increased strokes and/or TIAs risk. One or more of the specific APLAs tested were detected among 44% of patients. Therefore, these APLAs antibodies are considered independent risk factors for stroke and/or TIA events development related to APS. The roles played by aCL IgG with IgM, aβ2-GPI IgG, and aPS IgG and other parameters in inducing APS among the APLAs positive stroke and/or TIAs are more enhanced than among APS-DVT patients.

References:


