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## Prevalence of anti- beta2GPI antibodies and their isotypes in patients with renal diseases and clinical suspicion of antiphospholipid syndrome

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### ABSTRACT

**Background:** Antiphospholipid antibodies (aPL) are autoantibodies that are associated with a clinical state of hypercoagulability and diverse clinical manifestations collectively known as antiphospholipid syndrome (APS).

**Objectives:** To investigate the prevalence of anti-beta2glycoproteinI-antibodies (anti-β2GPI) and their isotypes in patients with renal diseases and clinical suspicion of antiphospholipid syndrome (APS).

**Patients and Methods:** This is a retrospective study in which we have analyzed the prevalence of anti-β2GPI and its isotypes in 170 patients on initial testing and in 29 patients repeated after 12 weeks for confirmation of APS. The clinical information was provided by the treating physicians or retrieved from the clinical records. The tests for anti-β2GPI screening and its isotypes (IgG, IgM and IgA) detection were assessed.

**Results:** On initial samples, anti-β2GPI was positive in 118 patients. IgA-β2GPI positivity (93; 79%) was significantly higher than IgM and IgG isotypes. Out of anti-β2GPI positive patients, clinical features in 95 patients were suggestive of APS or had SLE. Of these, IgA isotypes was found in 66% (P = 0.010), IgM in 31% (P = 0.033), and IgG in 11% (P = 0.033). On repeat testing, anti-β2GPI was persistently found in 22 patients with a continual predominance of IgA-anti-β2GPI over IgM and IgG isotypes (91% vs. 45.5% and 18% respectively).

**Conclusions:** Our results show that IgA-anti-β2GPI antibodies are the most prevalent isotypes in patients with renal disease or on renal replacement therapy in our population. Thus inclusion of IgA-anti-β2GPI in the testing repertoire may increase the diagnostic sensitivity for APS in patients with renal diseases.

### *Implication for health policy/practice/research/medical education:*

IgA-anti-β2GPI antibodies are the most prevalent antibodies in patients with renal disease or on renal replacement therapy with or without systemic lupus erythematosus in our population. The inclusion of these antibodies in testing repertoire for APS may result in better management in such patients.

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## 1. Background

**A**ntiphospholipid antibodies (aPL) are autoantibodies that are associated with a clinical state of hypercoagulability and diverse clinical manifestations collectively known as antiphospholipid syndrome (APS). These autoantibodies are also found transiently in acute and chronic infections, malignancies and other inflammatory conditions. The most frequently tested aPL are anticardiolipin-antibodies (aCL) and anti-beta2GPI-antibodies (anti-β2GPI) (1- 5).

According to the last updated classification criteria for APS, the clinical features include manifestations of arterial or venous thrombosis and fetal loss. The laboratory criteria include persistent positivity of lupus anticoagulants (LA) and/or IgG or IgM isotypes of aCL or anti-β2GPI. LA is detected by prolongation in coagulation assays, while the later two aPLs are detected by enzyme linked immunosorbent assay (ELISA) (1, 5, 6).

The other features that have been reported for APS but not included in the classification criteria are livedo reticularis, microangiopathy, thrombocytopenia, organ or tissue infarction, renal APS and others (1, 3, 7). Patients with renal disorders especially glomerulonephritis or other renal diseases may have concomitant autoimmune disease such as systemic lupus erythematosus (SLE). Such patients are also at risk of developing APS and may show heterogeneous manifestations (1, 3, 7-12). Moreover APS is also considered as one of the causes of recurrent failures of arteriovenous fistula formation (AVF) in candidates for renal transplantation. Catastrophic APS may occur at the time of transplant surgery resulting in acute graft loss (8, 10, 12, 13).

Clinicians tend to request test with which they are familiar and feel comfortable in their clinical interpretation. Devreese and Hoylaerts stated in their review article that most of the physicians

dealing with APS are not comfortable in the interpretation of aPL results as they are unfamiliar with the panel of tests' reports [2]. Among the laboratory parameters for APS classification, IgA isotypes of aPL are not included in the diagnostic criteria, mainly because most of the studies that have reported the prevalence of anti-β2GPI isotypes in APS either have not tested for IgA isotypes or show variable results [1, 3, 10, 12]. This sometimes creates uncertainty in the diagnosis and management of this syndrome as the presence of isolated IgA isotypes is not considered sufficient to diagnose APS. Consequently treating physicians usually do not request for repeat testing after 12 weeks as proposed in the guidelines for confirmation of APS.

It has been shown that anti-β2GPI are the most sensitive and specific markers in diagnosing APS (4, 5, 13, 14). IgG and IgM -anti-β2GPI are included in the classification criteria but the role of IgA-anti-β2GPI is still controversial (1, 2, 5, 12, 15-20).

## 2. Objectives

To investigate the diagnostic significance of anti-β2GPI isotypes in our population we carried out a retrospective analysis of laboratory and clinical data of patients who were tested for these autoantibodies at our centre. Majority of these patients suffered from renal diseases or were on renal replacement therapy (hemodialysis or renal transplant recipients). Complications including recurrent arterio-venous-fistula (AVF) failures and catastrophic APS warrant identification of sensitive and specific laboratory markers for diagnosing APS.

## 3. Patients and Methods

This is a retrospective study in which we have analyzed the prevalence of anti-β2GPI and its isotypes in 170 patients (between January 2006

and October 2009). The test for aPL was requested in these patients for suspicion of APS, or ordered as a part of routine screening in patients with SLE (diagnosed according to revised ACR criteria (21). In few of these patients, the clinical features were not in favor of diagnostic workup for APS.

The samples were received from nephrology, hemodialysis and transplant units. The clinical information was provided by the treating physicians or retrieved from the clinical records. The presence of infections and malignancies were ruled out by the treating physicians in all these patients. Majority of anti-β2GPI positive samples showed presence of IgA isotypes. A repeat sample was sent for only 29 patients after 12 weeks for confirmation of APS. We did a clinical and laboratory data analysis initially of all 170 patients requested for aPL and sub analysis of these 29 patients to find out the importance of IgA-anti-β2GPI in our patient population.

In APS related manifestations, symptoms asso-

ciated with hypercoagulability, pregnancy morbidity, AVF failures and other features as described previously, were included (1-3, 7-9, 22).

The tests for anti-β2GPI screening and its isotypes (IgG, IgM and IgA) were done by enzyme linked immunosorbent assay (ELISA) using commercially available kits (Binding Site, Birmingham, UK). Anti-β2GPI isotype titers were arbitrarily grouped into low ( $\leq 40$  IU/ml) and moderate to high ( $\geq 41$  IU/ml).

The data was analyzed using SPSS software version 12 (Chicago, IL, USA). Quantitative measurements are expressed as mean  $\pm$  SD and categorical variables are expressed in percentages.  $\chi^2$  or Fisher's exact tests and student's t-test were used to calculate the significance of differences in categorical and scalar variables wherever appropriate.  $P \leq 0.05$  was considered significant.

#### 4. Results

The mean age of all patients was  $28 \pm 11$  years. There were 109 (64%) females and 61 (36%)

**Table 1.** Prevalence of Anti-β2GPI Isotypes in Association with Clinical Features in all Patients.

Symptoms	anti-β2GPI n (%)	anti-β2GPI -IgA n (%)	anti-β2GPI -IgM n (%)	anti-β2GPI -IgG n (%)	P1	P2	P3
APS <sup>1</sup> and/or SLE <sup>2</sup> (n = 132)	95 (72)	87 (66)	41 (31)	14 (11)	0.010	0.033	0.022
Non-specific (n = 38)	23 (60.5)	21 (60.5)	5 (22)	2 (9)	0.000	0.136	0.509
Thrombotic and ischemic complications (n = 47)	32 (68)	29 (91)	11 (34)	6 (19)	0.000	0.009	0.157
Pregnancy complications <sup>3</sup> (n = 31)	25 (81)	24 (96)	10 (40)	4 (16)	0.000	0.141	0.561
Renal involvement <sup>4</sup> (n = 25)	17 (68)	16 (94)	8 (47)	3 (18)	0.000	0.026	0.527
AVF <sup>5</sup> failure (n = 24)	15 (62.5)	12 (80)	7 (47)	0	0.000	0.022	**
Cerebral vessel thrombosis or infarction/ ischemia (n = 13)	9 (69)	8 (89)	3 (33)	1 (11)	0.007	0.497	1.000
Livedo reticularis (n = 12)	11 (92)	10 (91)	4 (36)	2 (18)	0.167	1.000	1.000
Digital infarction (n = 7)	5 (71)	4 (86)	2 (40)	2 (40)	0.143	0.000	0.000
Thrombocytopenia (n = 3)	2 (67)	2 (100)	0	0	**	**	**
Raynaud's phenomenon (n = 1)	1 (100)	1 (100)	1 (100)	1 (100)	**	**	**

<sup>1</sup>= antiphospholipid syndrome, <sup>2</sup>=systemic lupus erythematosus, <sup>3</sup>=include fetal loss in 26 and pre-eclampsia in five patients, <sup>4</sup>=renal involvement consistent with APS, <sup>5</sup>=arterio-venous fistula formation, P1, P2, P3 = significant P values for IgA, IgM and IgG isotypes calculated from anti-β2GPI positive results, \*\* = P could not be computed due to very small number.

males. There was no difference in the mean age of patients positive or negative for anti- $\beta$ 2GPI. However these antibodies were found in a significantly higher number of female patients compared to males (83; 76% and 35; 57% respectively,  $P = 0.013$ ). On initial samples, anti- $\beta$ 2GPI was positive in 118(69%) patients. IgA- $\beta$ 2GPI positivity (93; 79%) was significantly higher than IgM and IgG isotypes (46; 39%,  $P = 0.007$  and 16; 14%,  $P = 0.006$  respectively). IgG-anti- $\beta$ 2GPI positivity was also significantly lower than IgM isotypes ( $P = 0.002$ ). Out of anti- $\beta$ 2GPI positive patients, clinical features in 95 patients were suggestive of APS or had SLE. While in 23 patients symptoms were not consistent with APS neither they had SLE. The distribution of anti- $\beta$ 2GPI isotypes in relation to clinical features in all patients are shown in table 1.

Of 118 anti- $\beta$ 2GPI positive patients, Isolated IgA was present in 65 (55%), isolated IgM in 9 (7.6%), isolated IgG in 1(0.8%) and mixed isotypes in 43 (IgA and IgM in 28; 24%, IgA and IgG isotypes in 6; 5% and all three isotypes in 9; 7.6%) patients. The breakup of the distribution of these antibodies in patients with and without APS related symptoms and/or SLE are shown in table 2. Overall there was no significant difference in the levels of IgA, IgM and IgG isotypes in patients with or without APS specific symptoms.

Repeat testing was done with fresh samples in 29 patients at a mean of  $48 \pm 46$  weeks (median = 32 weeks). Of these 22 (76%) had persistent positivity for anti- $\beta$ 2GPI. IgA was repeatedly positive in 20 (91%) patients ( $P = 0.000$ ), IgM isotypes in 10 (45.5%,  $P = 0.063$ ) and IgG in 4 (18%,  $P = 0.546$ ). The distribution of anti- $\beta$ 2GPI isotypes in relation to clinical features after repeat testing is shown in table 3. Eleven (50%) of these patients had isolated IgA and nine (41%) patients had more than one isotypes with IgA and IgM in

six (27%) and IgA, IgM and IgG- $\beta$ 2GPI in three (14%). Isolated IgG and IgM were present in 1 patient each.

**Table 2.** Comparison of Distribution of Anti- $\beta$ 2GPI Isotypes in Patients with Specific and Non-Specific Symptoms.

Anti- $\beta$ 2GPI Iso-types	APS1 related symptoms + SLE <sup>2</sup> (n =95), n (%)	Non Specific symptoms (n = 23) n (%)
-IgA	48 (51)	17 (74)
-IgM	7 (7)	2 (9)
-IgG	1 (1)	0
IgA + IgM	26 (27.3)	2 (9)
IgA + IgG	5 (5.3)	1 (4)
IgA + IgM + IgG	8(8.4)	1 (4)

<sup>1</sup>= antiphospholipid syndrome, <sup>2</sup>=systemic lupus erythematosus

### Anti- $\beta$ 2GPI and SLE

Of total, 121 patients were evaluated for SLE (according to the revised ACR criteria<sup>21</sup>) and 48 (40%) were diagnosed as having these disease. Anti- $\beta$ 2GPI was present in 41 (85%;  $P = 0.081$ ) with IgA isotypes in 38 (93%;  $P = 0.000$ ), IgM in 20 (49%;  $P = 0.032$ ) and IgG in 6 (15%;  $P = 0.573$ ) patients.

On repeat testing anti- $\beta$ 2GPI was found in 11/13(85%;  $P = 0.642$ ) SLE patients with IgA isotypes in 10 (91;  $P = 0.038$ ), IgM in 7(64%; 0.192) and IgG in 2(18%;  $P = 1.000$ ). Lupus nephritis (histopathology proven) was present in seven of these patients. APS features coexisted in 8 (73%) of these repeatedly positive anti- $\beta$ 2GPI SLE patients. The features included livedo reticularis (four), thrombotic and ischemic manifestations (three), APS renal involvement (two), thrombotic manifestations of central nervous system (two), pregnancy loss and AVF failure (one each). Two of these patients had worsening of renal functions. Both were positive for IgA- $\beta$ 2GPI and one was also positive for IgM isotypes.

**Table 3.** Prevalence of Anti-β2GPI isotypes on repeat testing in Association with Clinical Features in 22 patients.

Symptoms	anti-β2GPI n (%)	anti-β2GPI-IgA n (%)	anti-β2GPI-IgM n (%)	anti-β2GPI-IgG n (%)	P1	P2	P3
Thrombotic and ischemic complications (n = 10)	8 (80)	7 (87.5)	5 (62.5)	3 (37.5)	0.067	0.444	1.000
Pregnancy complications <sup>1</sup> (n = 4)	4 (100)	4 (100)	0	0	**	**	**
Renal involvement <sup>2</sup> (n = 4)	4 (100)	4 (100)	2 (50)	0	**	**	**
AVF <sup>3</sup> failure (n = 6)	3 (50)	3(100)	2 (67)	0	0.100	0.400	**
Cerebral vessel thrombosis or infarction/ ischemia (n = 3)	2 (69)	2 (100)	2 (100)	1 (50)	0.333	0.333	1.000
Livedo reticularis (n = 5)	5 (100)	5 (100)	3 (60)	2 (40)	**	**	**
Digital infarction (n = 1)	1 (100)	1 (100)	1 (100)	1 (100)	**	**	**

<sup>1</sup>=include fetal loss in three and pre-eclampsia in one patient, <sup>2</sup>=renal involvement consistent with APS, <sup>3</sup>=arterio-venous fistula formation, P1, P2, P3 = significant P values for IgA, IgM and IgG isotypes calculated from anti-β2GPI positive results, \*\*=*P* could not be computed due to very small number.

**Table 4.** Renal Manifestations of APS1 with anti-β2GPI Positivity.

APS Manifestations	anti-β2GPI n (%)	anti-β2GPI-IgA n (%)	anti-β2GPI-IgM n (%)	anti-β2GPI-IgG n (%)
<b>On Initial Testing (n = 17)</b>				
APS nephropathy <sup>2</sup> (n= 7)	4 (57%)	4 (100)	2 (50)	0
RAS <sup>3</sup> (n = 7)	5 (71)	5 (100)	1 (100)	0
FSGS <sup>4</sup> (n = 6)	4 (67)	3 (75)	2 (50)	1 (25)
Renal infarction (n=3)	2 (67)	2 (100)	1 (50)	1 (50)
TMA <sup>5</sup> (n=3)	3 (100)	3(100)	2 (67)	1(33)
Renal vessel thrombosis (n=3)	1(33)	1 (100)	1 (100)	0
<b>On Repeat Testing (n = 4)</b>				
APS nephropathy <sup>2</sup> (n=2)	2 (100)	2 (100)	1 (50)	0
RAS <sup>3</sup> (n = 1)	1 (100)	1 (100)	1 (100)	0
TMA <sup>5</sup> (n = 2)	2 (100)	2 (100)	1 (50)	0

<sup>1</sup>=antiphospholipid syndrome, <sup>2</sup>=constellation of hypertension, proteinuria, hematuria and renal insufficiency, <sup>3</sup>= renal artery stenosis,

<sup>4</sup>= focal segmental glomerulosclerosis, <sup>5</sup>=thrombotic microangiopathy

### Anti-β2GPI and Renal Manifestations

Renal manifestations (consistent with clinically suspected APS as previously described (7-9, 22) and diagnosed by laboratory features, renal histology and imaging) were present in 17 anti-β2GPI positive patients on initial testing. The isotype distribution is shown in table 1. The test was repeated in only four of these patients with 100% positivity for anti-β2GPI and its IgA isotype. IgG was not found in these patients on repeat testing (table 3). The various renal APS manifestations on initial and repeat testing are shown in table 4.

### Anti-β2GPI and AVF failure

Around 50% of patients, who were on maintenance hemodialysis and had AVF failure, were positive for anti-β2GPI on initial as well as repeat testing. IgA was present in 80% patients' samples initially and in 100% on repeat testing. Interestingly IgG was not found in these patients (table 1 & 3).

Three transplant recipients with anti-β2GPI positivity died of intracranial bleeding. One of these patients was a case of SLE and had a previous history of recurrent thrombosis with repeti-



tively high titers of IgA and IgG isotypes. The other two patients in whom the test was not repeated had IgA in high titers and IgM and IgG in low titers.

## 5. Discussion

The heterogeneity of aPL and variations in the clinical presentation of patients with APS indicate the complexity in the pathogenesis of this syndrome (2, 3, 5, 7-9). Similarly the ethnicity and environment have an influence on the prevalence of various aPL (5, 7, 12, 13, 15-20).

The anti- $\beta$ 2GPI is considered to be the most important and superior aPL for diagnosing APS (4, 5, 10, 13, 19, 20). IgG and IgM isotypes of anti- $\beta$ 2GPI have been included in the classification criteria (1, 2, 8, 9). However, paucity of the available data has not allowed the inclusion of IgA-anti- $\beta$ 2GPI in the last updated classification criteria for this syndrome (1, 12-14, 16-18). Existing data indicates that IgA- $\beta$ 2GPI is more frequent in Afro-American and Afro-Caribbean populations than Caucasians and Asians (13, 15, 16, 19). In the European population, this isotype does not seem to have a role in the APS diagnosis (17). The patient population in the present study comprised of mixed ethnicities of Indo-Pak Subcontinent. The significant finding in this study is the isolated prevalence of IgA isotypes in patients with APS related symptoms with or without SLE. Previous studies that have found significant association of IgA- $\beta$ 2GPI isotypes in their populations have also reported concurrent occurrence of IgM isotypes (15) or showed a significant association of IgA isotypes with APS in SLE patients only (12, 20). The IgG-anti- $\beta$ 2GPI was found at low frequency in this study population, similar to the findings of studies done in Korean and Chinese populations (6). These differences, which signify variations in the immune

response due to geographical and ethnical diversities (5, 6, 12, 15, 16, 20) underscore the importance of IgA- $\beta$ 2GPI as an independent parameter to diagnose APS.

The prevalence of IgA-anti- $\beta$ 2GPI isotypes in this study was found comparable in both sets of patients with or without APS related symptoms and/or SLE. This may lead to the arguments on the clinical utility of IgA-anti- $\beta$ 2GPI in the diagnosis of APS. Nevertheless it should also be noted that in APS symptomatic and SLE patients IgA was the only isotype persistently present in a greater number of patients compared to isolated IgG and IgM isotypes even on repeat testing. Hence disregarding the positivity of IgA-anti- $\beta$ 2GPI isotypes may decrease the sensitivity of laboratory parameters for diagnosing APS.

The depressed immune response due to uremia results in poor correlation of aPL with APS in hemodialysis patients (22-28). However it is reported that aPL significantly increases the risk of AVF failure in renal failure patients and is associated with increased surgical complications and graft loss after transplantation (8, 11, 22, 24-28). Most of the earlier studies that investigated the association of aPL with AVF failure tested for aCL only, and reported the prevalence of IgG-aCL between 4-50% (11, 22, 24, 28). Canaud et.al (11) have included anti- $\beta$ 2GPI in their testing repertoire but omitted IgA-anti- $\beta$ 2GPI and did not find an association between anti- $\beta$ 2GPI and AVF failure. We found the prevalence of anti- $\beta$ 2GPI in around 50% of patients with AVF failure on initial as well as repeat testing. The IgA- $\beta$ 2GPI was present in around 100% of these patients while IgG was completely absent in these patients. This again emphasizes the need to include anti- $\beta$ 2GPI with its all three isotypes to diagnose APS in patients with AVF failure to avoid later complications (8, 11, 22, 24-28).

Earlier studies have reported the prevalence of aPL in SLE patients to be around 13-60%, with up to 70% symptomatic for APS (4, 6-8, 12). The renal involvement of APS in these patients is reported to be around 8.7% to 40% (3, 7-9, 23). We found anti- $\beta$ 2GPI in 85% of SLE patients with 91% positivity for IgA isotypes on repeat testing. APS was present in around 73% of SLE patients in this study, but only 18% of these patients had APS specific renal involvement. As reported previously (3, 8, 9-11), we also found that anti- $\beta$ 2GPI add to the severity of disease in SLE patients with renal involvement and IgA as the major isotype present in these patients.

One of the proposed mechanisms of APS pathogenesis involves complement activation resulting in up regulation of adhesion molecules and cytokines secretion. This causes activation of endothelial cells and thrombus formation (14, 15, 30, 31). IgA antibodies can activate complements either by alternate or mannose binding lectin (MBL) pathways. Also abnormal glycosylation of IgA antibodies is associated with immune dysregulation (32, 33). Perhaps these factors may be contributing in the mechanism of APS pathogenesis in patients with IgA-anti- $\beta$ 2GPI positivity. However further investigations are required to elucidate the role of these antibodies and complement activation pathways in the pathogenesis of APS. This will give us further insight into the mechanism of the disease and may help in better patient management (31).

In this study repeat testing was not requested on majority of patients by treating physicians, perhaps due to lack of awareness about the importance of IgA- $\beta$ 2GPI positivity. It is important to note that on repeat testing, almost the same pattern of anti- $\beta$ 2GPI isotypes emerged. In fact the IgG isotypes became absent in patients with renal APS manifestations and pregnancy mor-

bidity. Therefore omitting IgA isotypes from the testing repertoire and only relying on the positivity of IgG or IgM may decrease the sensitivity of the assay system to diagnose APS.

Our institute is the largest nephrology, urology and transplant centre in the public sector catering to a huge number of patients with renal diseases across the country. The patients included in this study were therefore either being managed for renal diseases or were on renal replacement therapy. The catchment area of our institute comprises mostly of Southern Pakistan but we receive patients from all over the country. We believe that our results are fairly representative of the true prevalence of these antibodies and their isotypes for the whole population. However, there are certain limitations in the study. These include its retrospective nature, relatively small size, the patient population comprised of patients with renal diseases and a lack of repeat testing in a majority of patients. To look at the patterns of distribution of various isotypes of these antibodies in renal and non-renal patients, a larger generalized prospective study is required.

## 6. Conclusions

In conclusion, high prevalence of IgA- $\beta$ 2GPI antibodies with APS in this analysis indicates that this isotype has a role in the diagnosis of APS. This is also highlighted by the fact that anti- $\beta$ 2GPI positive transplant recipients who expired due to APS complications had high IgA isotype titers. Thus all patients with SLE or on hemodialysis with clinical features suggestive of APS should be screened for aPL.

Moreover it is important to investigate and validate biomarkers that may help in eliminating the need to repeat test after 12 weeks for confirmation of APS.

## Conflict of interest

None to declare

## Financial interests

None to declare

## Authors' contributions

SA designed the research and prepared the primary draft and the final manuscript. EA and RM had equal contribution in providing extensive intellectual contribution.

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