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To study the concentration of anti-B2GPI (IgG and IgMisotypes) in patients with collagen vascular diseases (auto-immune disorders), pregnancy related complication and thromboembolic disorders

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Abstract:

Background&Method: This is a retrospective study carried out with an aim to study the concentration of anti-B2GPI (IgG and IgMisotypes) in patients with collagen vascular diseases (auto-immune disorders), pregnancy related complication and thromboembolic disorders, in patients who were tested for anti-B2GPI (IgG and IgM) and ACL (IgG and IgM) by the standardized solid-phase enzyme linked immunosorbent assay (ELISA) in pathology department.

Result: The 38 patients with SLE presented with common clinical manifestations of SLE. In addition, Raynaud's phenomenon (n = 3) and photosensitivity (n = 4) were seen in a few. Further, two each of patients with SLE had auto-immune hemolytic anemia, and history of spontaneous abortions respectively. One of the two patients with spontaneous abortions had a past history of a vertebrobasilar infarct. Renal involvement was noted in 13 (34.2%) cases, out of which 11 had biopsy proven lupus nephritis, one case had mild mesangial proliferation and in one ultrasound of the abdomen had shown medical renal disease. One patient had a clinical diagnosis of deep vein thrombosis however results of radiological tests for confirmation if done were not available. One other patient had pulmonary hypertension. The patient with primary APS had presented with seizures and post-ictal confusion.

Conclusion: In present study ACL (IG & or IgM) was noted to be more prevalent than anti-B2GPI (IG & or IgM) in patients with collagen vascular diseases (auto-immune diseases) and thromboembolic disorders while they showed a similar prevalence in patients with recurrent fetal losses. While we observed that there were certain differences in the prevalence of these antibodies when compared to other published studies, this could be related to differences in patient selection.

Keywords:anti-B2GPI, collagen, pregnancy & thromboembolic.

Study Designed: Retrospective Study.

1. INTRODUCTION

Antiphospholipid (aPL) antibodies are a heterogeneous group of immunoglobulins that have been associated with various manifestations of the antiphospholipid syndrome (APS). They include anticardiolipin antibodies (ACL), anti-beta 2 glycoprotein I antibodies (Anti-B2GPI), lupus anticoagulants (LA) and others (such as antiphosphatidyl-serine, antiphosphatidyl inositol, antithrombin antibodies etc.).(1)

A variety of clinical manifestations have been observed in patients with aPL antibodies, while various thromboembolic disorders can occur in such patients; in pregnancy these antibodies can also cause recurrent fetal losses and other pregnancy related complications.(2) The syndrome can present as primary disorder (Primary APS) or may be associated with other autoimmune diseases most often SLE (Secondary APS). (3)

However, the criteria for the laboratory diagnosis of APS remain controversial. It has been reported in some studies that anti-B2GPI are more specific than ACL and therefore the laboratory criteria should be limited to anti-B2GPI only and testing for ACL should be omitted.(4) Nevertheless certain other studies have proposed that at present it is important to continue testing for anti-B2GPI as well as ACL for the diagnosis of APS and also that low-titre ACL and anti-B2GPI should be considered.(5) Certain studies have also observed differences in the aPL profiles between patients with thromboembolism related APS and obstetric complications related APS.(7) Thus while the international criteria for APS are termed classification criteria intended mainly for research studies their application in the diagnosis of APS is fraught with challenges.

The antiphospholipid syndrome (APS) was first described in the 1980s, studies at the London Hammersmith Hospital led to the development of solid-phase radioimmunoassay to detect ACL and a high correlation was documented between the IgG ACL and thrombosis.(8) These findings led to the recognition of the so-called anticardiolipin syndrome. Later in 1985 the first quantitative ELISA to identify various isotypes (usually IgG, sometimes IgM, and rarely IgA) of antibodies directed to phospholipids or their cofactors was developed by Harris and coworkers.(9) Subsequently these antibodies were also found to react with other phospholipids, so the name changed from primary ’anticardiolipin syndrome’ to ’anti-phospholipid syndrome’. APS was first recognized in patients with systemic lupus erythematosus (SLE) and found in patients with other autoimmune disorders in lower frequency.

2. MATERIAL & METHOD

This is a retrospective study carried out in patients who were tested for anti-B2GPI (IgG and IgM) and ACL (IgG and IgM) by the standardized solid-phase enzyme linked immunosorbentassay (ELISA) in our department of SETH GSMC AND KEMH, Mumbai, Maharashtrabetween December 2016 to September 2017.

STUDY PARTICIPANTS

A total of 130 patients were enrolled for this study.

The study subjects were categorized into 3 disease groups:

1. Collagen vascular / auto-immune diseases (n=47)
2. Thromboembolic disorders (n=46)

3. Pregnancy related complications (n=37)

All available data of demographic parameters like age and gender, clinical manifestations including obstetric history, other laboratory tests and radiological investigations that were documented in records were reviewed.

INCLUSION CRITERIA:

All patients with collagen vascular / auto-immune diseases, pregnancy related complications and thromboembolic disorders who were tested for anti-B2GPI as well as ACL of both IgG and IgMisotypes in the same blood sample, in the above-stated time period, by using standardized ELISA kits and in whom clinical information is available.

EXCLUSION CRITERIA:

1. Patients tested for anti-B2GPI and ACL of both IgG and IgMisotypes in the same blood sample by standardized ELISA but in whom adequate clinical data is not available.
2. Patients tested for the abovementioned antibodies but who do not have any documented features of collagen vascular / auto-immune diseases, pregnancy related complications and thromboembolic disorders

3. METHODOLOGY

Blood samples: Venous blood had been collected from patients for performing the tests in non-anticoagulatedvacutainers. This project was approved by the Institutional Review Board of this institute. Strict adherence to the ethical principles of the Declaration of Helsinki was ensured.

Test procedure: The estimation was performed using standardized, commercial ELISA kits that included polystyrene ELISA microplates with 96 wells precoated with antigen, anti-human IgG and IgM enzyme-linked conjugates, calibrators, controls, buffers and diluents for anti-B2GPI-IgG and anti-B2GPI-IgM (Imtec, Germany) and ACL-IgG and ACL-IgM (Genesis Diagnostics, UK). The antigen used for coating the ELISA plates were purified human beta-2 glycoprotein I for estimation anti-B2GPI-IgG and anti-B2GPI-IgM and purified cardiolipin configured with beta-2-glycoprotein I as a cofactor for estimation of ACL- IgG and ACL-IgM. The measurements were taken at 450 nm using an automatedELISA Reader (Biotech ELx808). The levels of antibodies were derived from a computerized standard curve.

For ACL concentrations greater than 11 GPLU/ml for ACL-IgG and greater than 10 MPL/ml for ACL-IgM were considered as positive according to the cut- offs mentioned in the instruction pamphlet supplied with the ELISA kits. Similarly, for anti-B2GPI concentrations greater than 7 U/ml for IgG and IgM type antibody were considered to be positive. However, in accordance with the Sapporo classification criteria for APS, concentrations greater than 40 GPLU/ml and 40 MPLU/ml were considered to be moderate to severe elevations.

4. RESULTS

Table 1: Distribution of cases

Study group	**No. of cases**	**Percentage**
Collagen Vascular Diseases (Autoimmune diseases)	47	36.15%

Thromboembolic disorders	46	35.48%
Recurrent fetal losses	37	28.46%
Total	130	100%

Among the 47 patients with collagen vascular diseases (autoimmune diseases) the majority were patients with SLE (n = 38), while the remainder comprised Scleroderma (n = 2), autoimmune hemolytic anemia (n = 2), Rheumatoid arthritis (n = 1), Rheumatoid arthritis with possibly Scleroderma overlap (n = 1), Sjogren's syndrome (n = 2), and primary APS (n = 1).

Table 2: Distribution of cases according to age

Age range	**No. of cases**	**Percentage**
< 20 years	10	7.7%
21-30 years	49	37.7%
31-40 years	43	33.1%
41-50 years	22	16.9%
51-60 years	6	4.6%
Total	130	100%

Table 3: Clinical manifestations in SLE (n=38)

Complaint	**No. of cases**	**Percentage**
Joint pain	24	63.1%
Hair loss	15	39.4%
Oral ulcer	12	31.5%
Malar rash	3	7.8%

Fever	10	26.3%
Renal disease	13	34.2%
Raynaud's phenomenon	3	7.8%
Photosensitivity	4	10.5%
Autoimmune hemolytic anemia	2	5.2%
Spontaneous abortions	2	5.2%
Pulmonary hypertension	1	2.6%

Table 4: Clinical manifestations in thromboembolic diseases (n=46)

Complaint	**No. of cases**	**Percentage**
Deep venous thrombosis	19	41.3%
Arterial thrombosis	6	13%
Limb swelling	13	28.2%
Weakness	8	17.3%
Chest pain	8	17.3%
Vomiting	7	15.2%
Breathlessness	6	13%
Dyspnoea on exertion	5	11%
Palpitation	5	11%
Headache	5	11%
Abdominal pain	5	11%

Blackish discoloration of skin	2	4.3%

The 38 patients with SLE presented with common clinical manifestations of SLE. In addition, Raynaud's phenomenon (n = 3) and photosensitivity (n = 4) were seen in a few. Further, two each of patients with SLE had auto-immune hemolytic anemia, and history of spontaneous abortions respectively. One of the two patients with spontaneous abortions had a past history of a vertebrobasilar infarct. Renal involvement was noted in 13 (34.2%) cases, out of which 11 had biopsy proven lupus nephritis, one case had mild mesangial proliferation and in one ultrasound of the abdomen had shown medical renal disease. One patient had a clinical diagnosis of deep vein thrombosis however results of radiological tests for confirmation if done were not available. One other patient had pulmonary hypertension. The patient with primary APS had presented with seizures and post-ictal confusion.

The patients with thromboembolic diseases comprised 38 cases of radiologically proven venous thrombosis. These included deep vein thrombosis (DVT) (n = 19) out of which 7(15.2%) patients had documented pulmonary thromboembolism (PTE). The other sites of venous thrombosis were cortical venous (n =3), portal veins (n = 2) and in one case each in the dural sinuses, cavernous sinus, inferior jugular vein, axillary with subclavian veins, brachiocephalic vein, cephalic vein, and inferior mesenteric vein. The other patients in the thromboembolic group were 6 cases of radiologically proven arterial thrombosis (one each in the abdominal aorta, brachial, femoral, popliteal, carotid and ulnar-radial arteries). Two patients with brain infarcts were suspected to have thrombi in the posterior inferior cerebral artery and cerebellar vessels had no radiological documentation of the thrombi. The clinical manifestations in the group with thromboembolic diseases are depicted in Table.

As depicted in Table 7, patients with recurrent fetal losses (n = 37) had spontaneous abortions (n = 24; 64.8%), followed by vaginal bleeding (n = 12; 32.4%), fever (n = 4; 11%) and missed abortion (n = 3; 8.1%). Majority (n = 12) had two spontaneous abortions, followed by three (n = 7), four or five (n = 5) abortions. In addition, one patient was a known case of immune thrombocytopenic purpura.

5. DISCUSSION

In our study, the age range in these 130 cases was between 11-72 years, with majority of the patients i.e. 92 (70.7%) cases in the age group of 21-40 years. APS has an onset in the middle aged, i.e 30-40 years old, which is similar to our study.(15) The Euro-phospholipid cohort had around 10% patients who were older than 50 years and 2.5% below 15 years of age.(10)

Out of 130 cases, 102 cases were female (78.5%) and 28 cases were male (21.5%). This was in concordance with study by de Groot et al. in which about 80% of patients with APS were female(11). Data from clinical and experimental studies suggest that autoimmunity is affected by gender, immunoreactivity appears to be more pronounced in females than in males.(12)

In our study group of autoimmune diseases two SLE patients, each (5.2%), had auto- immune hemolytic anemia and a history of spontaneous abortions respectively, one patient each had a history of vertebrobasilar infarct and deep vein thrombosis while renal involvement was noted in 12 (31.5%). Nam et al.(13) studied 469 SLE cases, found hemolytic anemia in 8.5%, renal disorder in 36.9%, cerebrovascular disease in 10.1% and one spontaneous abortion in 9.4% cases.

In our study group of thromboembolic diseases, the patients with thromboembolic diseases comprised 38 cases of radiologically proven venous thrombosis. These included deep vein thrombosis (DVT) (41.3%) out of which 7 (15.2%) patients had documented pulmonary thromboembolism (PTE) and 6 (13%) cases of radiologically proven arterial thrombosis. Study by S Chandrashekhara et al.(14), showed deep vein thrombosis (DVT) in (29.8%) was the most common thrombotic condition, other sites of thrombosis were coronary artery (19.2%), central nervous system territory arteries (17.21%) and peripheral arteries (5.29%).
In our study group of recurrent fetal losses, (64.8%) patient had manifested with two or more fetal losses. Majority had two spontaneous abortions, followed by three, four or five abortions. This was in comparison with study by Sheela et al.(15), 84.8% patients had two previous miscarriages while 15.1% patients had three previous miscarriages.

6. CONCLUSION

In present study ACL (IG & or IgM) was noted to be more prevalent than anti-B2GPI (IG & or IgM) in patients with collagen vascular diseases (auto-immune diseases) and thromboembolic disorders while they showed a similar prevalence in patients with recurrent fetal losses. While we observed that there were certain differences in the prevalence of these antibodies when compared to other published studies, this could be related to differences in patient selection.

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