



# Detection of antiphospholipid syndrome in women who have had miscarriages Using anti-phosphatidylserine antibodies

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**Abstract:** *In this study, the results of the samples showed that: 21 Positive sample Due to the presence of antibodies to phosphatidylserine fats, the concentration rate in these positive cases reached  $(14.6 \pm 1.4$  RU/ml) in the patient group, while in the control group it did not. Positive cases appear and the average concentration of antibodies is  $(7.9 \pm 2.0$  RU/ml) in the group Control. The presence of these antibodies increased in the group of patients who had suffered three or more miscarriages and their number was 15 cases as samples the remaining 6 positives appeared in the group of women who had suffered two miscarriages, as they reached, The concentration rate of anti-phosphatidylserine antibodies in this group of patients  $(15.1 \pm 2.1$  RU/ml) and no positive result was shown in the group of women who were exposed. For one abortion.*

*And individuals reached 90 blood samples, 80 of which were collected from women who had been exposed. For cases of spontaneous or recurrent miscarriage and 10 collected from women with normal pregnancy As control samples, serum was prepared from these samples for use in screening for anti-Ps antibodies in patients.*

**Key Words:** *Antiphospholipid, miscarriage, autoimmune disorders, Neurological symptoms.*

## 1. INTRODUCTION:

Antiphospholipid syndrome is an autoimmune disease characterized by blood clots and complications during pregnancy, and is associated with the presence of antibodies to phospholipids. Interest in autoimmune factors as potential causes of recurrent miscarriage has increased significantly, especially after the identification of different types of antibodies and their association with these conditions. It is important to note that autoimmune disorders are more common in women, with an incidence rate of 6 to 10 times higher than in men, and these disorders often appear during the reproductive period.

Antiphospholipid syndrome (Antiphospholipid syndrome (APS) is an autoimmune disease characterized by blood clots and complications during pregnancy, accompanied by the presence of antibodies to phospholipids. Interest in autoimmune factors as causes of recurrent miscarriage (RPL) has increased significantly, especially after the discovery of different types of antibodies and their association with these conditions. It is worth noting that autoimmune disorders are common among women, ranging from 6 to 10 times that of men, and these disorders often appear at reproductive age.<sup>(1)</sup>

The current study aims to evaluate the role of anti-phospholipid antibodies of the phosphatidylserine type in the diagnosis of lipid syndrome.

## 2. Symptoms :

Signs and symptoms of antiphospholipid syndrome can include:

1. **Blood clots in the legs (deep vein thrombosis).** Signs of deep vein thrombosis include pain, swelling, and redness. These clots can travel to the lungs (pulmonary embolism).
2. **Recurrent cases of spontaneous abortion and stillbirth.** Other pregnancy complications include dangerously high blood pressure (preeclampsia) and premature birth.



3. **stroke.** Stroke can occur in a young person with antiphospholipid syndrome, but there are no known risk factors for cardiovascular disease.
4. **Transient ischemic attack (TIA)**. As with a stroke, a transient ischemic attack usually lasts only a few minutes and does not cause permanent damage.
5. **Rash.** Some people develop a red rash with a lacy, net-like pattern.
6. **Neurological symptoms.** Chronic headaches such as migraines, dementia, and seizures can occur when a blood clot blocks blood flow to parts of the brain.
7. **Cardiovascular disease.** Antiphospholipid syndrome can cause heart valve disease.
8. **Low platelet count.** This decrease in the blood cells needed for clotting can cause bleeding episodes, especially from the nose and gums. The bleeding appears on the skin as small red spots.(2)

## 2.1 Reasons

Antiphospholipid syndrome occurs when your immune system mistakenly produces antibodies that make your blood more likely to clot. Antibodies normally protect your body from foreign invaders, such as viruses and bacteria. Antiphospholipid syndrome can be caused by an underlying condition, such as an autoimmune disorder. It can also occur without an underlying cause.(3)

## 2.3 Risk factors

Women are more likely to develop antiphospholipid syndrome than men. Having another autoimmune disease, such as lupus, also increases your risk of developing antiphospholipid syndrome.(4)

It is possible to have antibodies associated with antiphospholipid syndrome (APS) without any signs or symptoms. However, the presence of these antibodies increases the risk of blood clots, especially in the following cases:

1. Pregnancy
2. Not moving for a period of time, such as staying in bed or sitting during a long flight
3. undergo surgery
4. Smoking
5. Taking oral contraceptives or treating menopausal problems with estrogen
6. High cholesterol and triglyceride levels.(5)

Antiphospholipid antibodies represent a group of heterogeneous antibodies that lead to thrombosis and complications during pregnancy, especially in cases of recurrent miscarriage. The most prominent antibodies are (APL) used in the diagnosis of the disease: Lupus Anticoagulant, Anticardiolipin Antibodies, and Anti-glycoprotein antibodies.(6)

In addition to the three antibodies mentioned, research has shown that there are other abnormal antibodies that play an important role in the occurrence of spontaneous abortions, such as antibodies to phosphatidylserine (Anti-Phosphatidyl Serine Antibodies).(7)

These antibodies are immunoglobulins that bind to phospholipids and plasma proteins that may interact with phospholipids on the surface of vascular cells or on platelets. Among the types of anti-phosphatidylserine antibodies that have been discovered, anti-Beta 2-Glycoprotein antibodies, Lupus anticoagulant, Anti coodiolipin antibodies, Lupus anticoagulant, and anti-coodiolipid antibodies are of great clinical importance, as they are of particular clinical and diagnostic importance in individuals at risk of thrombosis (P2GP1, LA, ACL) and women with recurrent pregnancy loss. There are also other types of autoantibodies that have not been fully identified, such as antibodies to phosphatidylserine, prothrombin, anxin, and phosphatidylinositol.(8)

An association has been found between autoantibodies and the clinical features of antiphospholipid syndrome (APS). A previous study has shown that anti-phosphatidylserine antibodies may affect the normal formation of the primary fetal membranes, which in turn affects the development of the placenta. This type of antibody was evaluated in a study that suggested an association between anti-phosphatidylserine antibodies and recurrent pregnancy failure, as well as patients with phospholipid syndrome. A significant association was also indicated between anti-phosphatidylserine antibodies and anti-phospholipid antibodies, especially when used in the diagnosis of cases that show



negative results for anti-phospholipid antibodies. Furthermore, anti-phosphatidylserine antibodies may show positive results in some women at risk of miscarriage, who showed negative results for anti-cardiac lipid antibodies.(9)

Anti-phosphatidylserine antibodies are a risk factor for recurrent miscarriage in women with preeclampsia, a study has shown. Therefore, measuring the level of these antibodies may help improve the condition of women with recurrent miscarriage who do not have other types of anti-phospholipid antibodies.<sup>(10)</sup>

### 3. Materials and working methods

It will be measured. Anti-phosphatidylserine antibody level using direct enzyme-linked immune sorbent assay

number	Quantity	The material
1	1.5 ml	enzyme-coupled antibodies
2	100 ml	Buffer solution for diluting samples
3	100 ml	Buffer solution for varnish
4	2.0 ml	Positive control sample
5	2.0 ml	Maltese control sample
6	2 ml	120 Ru/ml calibrator 1
7	2 ml	12 Ru/ml calibrator 1
8	2 ml	2 Ru/ml calibrator 1
9	12 ml	The solution of the material is the front
10	ml 12	Stop reaction solution

Table (1) shows the components Anti-phosphatidylcholine antibodies



.Figure (1) shows the device used

### 4. Principle of detection process

This kit allows the measurement of anti-phosphatidylserine antibody levels in serum or plasma, providing quantitative or semi-quantitative results. A polystyrene plate pre-coated with phosphatidylserine antigen is used, the activity of which with antibodies depends on the presence of the protein. Plasma GP1 as a cofactor for antigen recognition.

Therefore, the kit used contains this cofactor necessary for the reaction to occur. When the diluted serum containing the antibodies is added, these antibodies will react with the antigen, which may be of the type IgG, IgM, or IgA. To detect the formation of the antigen-antibody complex, enzyme-labelled anti-human IgG antibodies are added that bind to the complex (antigen/antibody) and induce a color reaction, where the intensity of the color is directly proportional to the concentration of antibodies present in the sample.



**How it works:**

First: We perform the dilution process for concentrated solutions:

- 1) We dilute the antibody solution 10:1 using the sample dilution buffer.
- 2) One volume of the buffer solution is added to 7 mL of distilled water to obtain a 10:1 diluted buffer solution.
- 3) 5 µl of serum is added to 1.0 ml of sample buffer solution and mixed well.

**Second: Steps**

- 1) The equipment is left for an hour to reach laboratory temperature.
- 2) I added 100 µl of water to each of the calibrators, positive and negative control samples, and serum samples in the wells and incubated the plate for 30 minutes at 18-25°C.
- 3) The wells were washed with 300 µl of diluted wash solution, and the solution was left in the wells for 90 s.
- 4) I added 100 µl of conjugated antibody solution to each well, and then incubated the plate for 45 min at 18-30°C.
- 5) The wells were washed with 280 µl of diluted wash solution, leaving the solution for 30-60 s.
- 6) 100 µl of the substrate solution was added to each well and the plate was incubated for 15 min at 18-25°C and then 100 µl of the stop solution was added to each well.

**5. Results and discussion:**

The results of the detection of antibodies to phosphatidylserine showed the presence of (21) positive cases for these antibodies in the patients’ samples, while the control samples did not show any positive results for this type of antibodies. The statistical analysis using the program (SPSS) There is a significant difference between the two groups at a significance level  $\leq 0.01$ .

and The average concentration of antibodies (Ph.s) Phosphatidy serine) In the group of patients (RU/ml  $1.4 \pm 14.6$ ), while the concentration in the control group was ( $7.9 \pm 2.0$ RU/ml), as shown in the table. (2).

Table 2: Number of samples positive for antibodies **Ph.S** In the patient group and the control group

PValue	Antibody concentration rate	%	Number of positive cases	%	Number of samples	Groups
0.01	1.4+14.6RU/ml	26.2	21	88.8	80	Patient group
	2.0+7.9RU/ml	0.0	0	11.1	10	control group

Positive cases of antibodies were distributed. Ph.s between groups according to the number of miscarriages as follows: In the group of women who had one miscarriage, no positive cases were recorded, and the mean concentration of these antibodies was RU/ml ( $1.4 \pm 8.8$ ). In the group of women who had two miscarriages, 6 positive cases were found with a mean concentration of antibodies of RU/m ( $15.1 \pm 2.1$  l).

While in the group of women who had three miscarriages, the highest number of positive cases was recorded, reaching 15 positive cases with an average concentration of RU/ml ( $1.1 \pm 14.4$ ). Statistical analysis showed a direct relationship between the presence of Ph.s antibodies and the number of miscarriages, as shown in Table 3 and Figure 4, which shows the relationship between the number of samples positive for Ph.s antibodies and the number of miscarriages, indicating an increase in the number of infections in women who had a greater number of miscarriages.

Table 3: Number of positive cases for antibodies **Ph.S** In patients and its relationship to the number of miscarriages

Antibody concentration rate	%	number Samples game	Antibody concentration rate	%	Number of samples positive for antibodies <b>Ph.S</b>	%	Number of samples	The opposite <b>Ph.S</b> Patient groups
1.4+8.8RU/ml	100	15	0.0	—	0	18.75	15	Set 1



2.5+8.0RU/ml	70	14	15.1+2.1RU/ml	30	6	25	20	Group 2
0.8+10.3RU/ml	66.66	30	14.4+1.1RU/ml	33.33	15	56.25	45	Group 3

- Group 1: Patients with a single miscarriage
- Group 2: Patients who had two miscarriages
- Group 3: Patients who had more than two miscarriages.

And a scientific study was recorded<sup>(11)</sup>, (52%) of patients who suffered from recurrent miscarriage and their number (34) A patient showed a positive result for the presence of Ph.s antibodies, and this study suggested Add antibody detection test Ph.S In conjunction With tests LA and ACA and  $\beta_2GB_1$  As it gives great importance to comprehensive examination to detect the syndrome. APS Special When it is a syndrome APS is primary.

The results of the current study showed that antibodies Ph.s appeared in (9) patients whose results were negative for the rest of the lipid antibodies. Phospholipidemia, this condition is forced into a condition called serologically negative samples for phospholipid syndrome. (Sero Negative (APS) Patients who have negative results for major anti phospholipid antibodies are: (ACA and Ia and B2GP1) but they have symptoms Clinical confirmation of the disease and positive results for atypical antibodies for diagnosis APS like opposites Ph.S and A joint study conducted between the United States concluded that American Britain that patients suffering from clinical manifestations of the syndrome APS but they are negative for the evidence. Classical they may be subject to tests. Additional not Classical

Another study (12), which included (872) women suffering from : RPL, that (49) cases had negative results for the presence of LA and ACA antibodies, but they showed a positive result for the presence of Ph.s antibodies. They also concluded in this study that the number of cases positive for the presence of Ph.s antibodies was directly associated with the number of miscarriage cases.

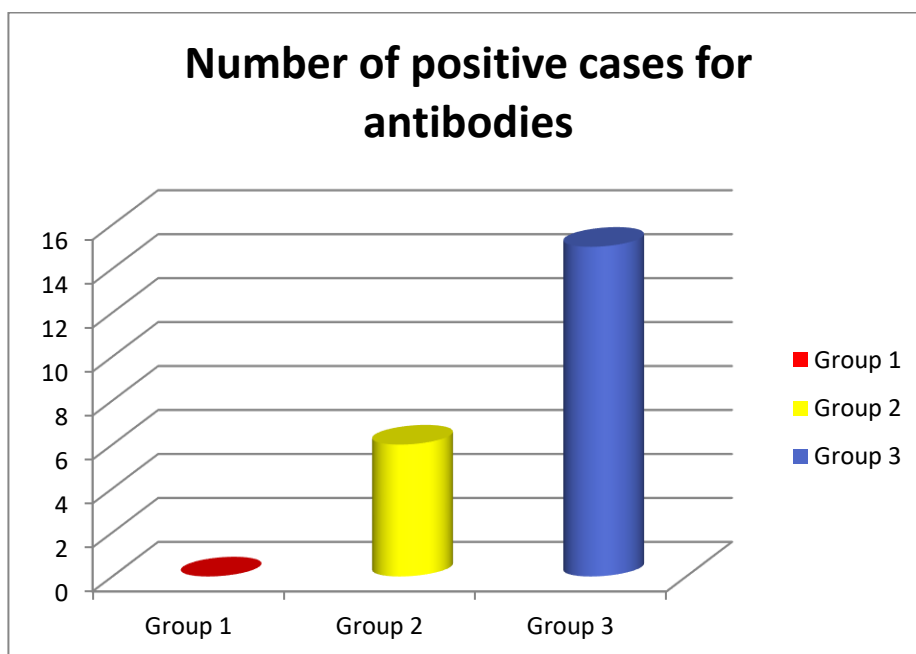


Figure 2: Number of positive cases in the patient group and its relationship with the number of miscarriages

- Group 1: Patients with one miscarriage
- Group 2: Patients with two miscarriages
- Group 3: Patients with more than two miscarriages



**REFERENCES:**

1. Tebo, A.E.; Jaskowski, T.D.; Hill, H.R.; Branch, D. W. (2008). Clinical relevance of multiple antibody specificity testing in anti-phospholipid syndrome and recurrent pregnancy loss. *Clin. Experimental Immunol.*, 154(3), 332-338.
2. Velayuthaprabhu, S.; Archunan, G. (2005). Evaluation of anticardiolipin antibodies and antiphosphatidylserine antibodies in women with recurrent abortion. *Indian Med.Sci.*, 59(8), 347-352. <https://doi.org/10.4103/0019-5359.16651>
3. Zohoury, N.; Bertolaccini, M.L.; Rodriguez-Garcia, J.L.; Shums, Z.; Ateka-Barrutia, O.; Sorice, M.; Khamashta, M. (2017). Closing the serological gap in the antiphospholipid syndrome: the value of “non-criteria” antiphospholipid antibodies. *Rheumatol.*, 44(11), 1597-1602.
4. Whitaker KL Antiphospholipid antibody syndrome: The difficulties of diagnosis. *JAAPA*. 2017;30:10–14. doi: 10.1097/01.JAA.0000526771.67820.59 [PubMed]
5. 14.Khamashta MA Management of thrombosis and pregnancy loss in the antiphospholipid syndrome. *Lupus*. 1998;7:S162–S165. doi: 10.1177/096120339800700235 [PubMed]
6. Ruiz-Irastorza G., Crowther M., Branch W., Khamashta MA Antiphospholipid syndrome. *Lancet*. 2010;376:1498–1509. doi: 10.1016/S0140-6736(10)60709-X [PubMed]
7. 16. Miyakis S., Lockshin MD, Atsumi T., Branch DW, Brey RL, Cervera R., Derksen RHWM, De Groot PG, Koike T., Meroni PL, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS) *J. Thromb. Most.* 2006;4:295–306. doi: 10.1111/j.1538-7836.2006.01753.x.[PubMed]
8. Devreese KMJ, Ortel TL, Pengo V., De Laat B., Antibodies TSOLA Laboratory criteria for antiphospholipid syndrome: Communication from the SSC of the ISTH. *J. Thromb. Most.* 2018;16:809–813. doi: 10.1111/jth.13976. [PubMed]
9. Okuma, H.; Kitagawa. Y.; Takagi, S. (2010). Investigation of antiphosphatidyl-serine antibody and antiphosphatidyl-inositol antibody in ischemic stroke patients. *Clin, and Developmental Immunol.*, 2010, 439230. <https://doi.org/10.1155/2010/439230>
10. Pignatelli, p.; Ettore, E.; Menichelli, D., Pani, A.; Violi, F.; Pastori, D. (2020). Seronegative antiphospholipid syndrome: refining the value of “non-criteria” antibodies for diagnosis and clinical management. *Haematologica*, 105(3), 562-572.
11. Roggenbuck, D.; Egerer, K.; von Landenberg, p.; Hiemann, R.; Feist, E.; Burmester, GR; dbmer,
12. Khojeer, H.; Alfattani, A.; Al-Kaff, M.; Al Shehri, T.; Khojah, O.; Owaidah, T. (2015). Antiphosphatidylserine antibodies as diagnostic indicators of antiphospholipid syndrome. *Lupus*, 24(2), 186-190.
13. Jaslow, C.R.; Camey, J.L.; Kutteh, W. H. (2010). Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertility and sterility*, 93(4), 1234-1243.