Pereira, Karla N.; Beck, Sandra T.; Konopka, Cristine K.; Segala, Zanoni; Moresco, Rafael
N.; Silva, José E. P.

Level of reactivity of IgM anticardiolipin antibodies in preeclampsia: a likely early serologic
marker

Level of reactivity of IgM anticardiolipin antibodies in preeclampsia: a likely early serologic marker

Nível de reatividade de anticorpos IgM anticardiolipina na pré-eclâmpsia: um provável marcador sorológico precoce

Karla N. Pereira; Sandra T. Beck; Cristine K. Konopka; Zanoni Segala; Rafael N. Moresco; José E. P. Silva

Universidade Federal de Santa Maria (UFSM).

ABSTRACT

The purpose of this study was to measure the level of anticardiolipin antibodies in preeclamptic pregnant women to verify a possible association between the presence of these antibodies and the development of the syndrome. A total of 36 pregnant women with preeclampsia and 19 women with normal pregnancy were evaluated. Anticardiolipin antibodies were determined by enzyme immunoassay. The reactivity level of anticardiolipin antibodies was significantly higher in the preeclamptic group, with a significance level of 95%. The results can mean the participation of anticardiolipin antibodies in the pathogenesis of preeclampsia. Future studies may confirm this parameter as an early marker for the development of this disease.

Key words: preeclampsia; antiphospholipid; anticardiolipin; pregnancy.

INTRODUCTION

Hypertension disorders in pregnancy are considered the most common cause of maternal death in the whole world[1]. In Latin America, 25.7% of maternal and 18% of fetal deaths may happen as consequences of these disorders[2]. In Brazil, the rate of maternal mortality directly caused by eclampsia is 11.8%; specifically in the South region, the index is 7.18%[3]. The diagnosis of preeclampsia is determined by the presence of hypertension (systolic blood pressure (BP) ≥ 140 mm/Hg and diastolic BP ≥ 90 mm/Hg) and proteinuria ≥ 300 mg/24 h after the 20th week of gestation[4]. It is a multifactorial disease with genetic and environmental factors involved in its etiology[5]. Its variable clinical presentation and the absence of a single laboratory test to establish diagnosis sometimes make confirmation difficult[6].

In recent years, many efforts have been made to define physiopathological factors and possible screening methods to identify, before the onset of clinical signs or apparent symptoms, women who will eventually develop preeclampsia[7].

Antiphospholipid antibodies (APA) are a heterogeneous family of immunoglobulins class G (IgG), M (IgM) or A (IgA), with great affinity and specificity, and able to recognize several combinations of phospholipids, phospholipid-binding proteins (such as β2-glycoprotein-I [β2GPI] and prothrombin) or both. Platelets and endothelial cells are their major targets[8]. When these antibodies are associated with vascular thrombosis and pregnancy complications, like preeclampsia, recurrent pregnancy loss, restricted intrauterine growth and fetal death, they characterize the antiphospholipid antibody syndrome (APS)[9].

Studies that compared the levels of IgG subclasses of antibodies against β2GPI and cardiolipin showed association of IgG2 subclass with fetal losses and thrombosis[10]. IgG2 subclass was detected as the predominant IgG subclass against cardiolipin, characteristic of an immune response against non-protein antigens. In this kind of response, the stimulus for antibody production by B lymphocytes is directly provided, not aided by interleukins produced by auxiliary T lymphocytes[11]. As a result, the produced immunoglobulins are preferably IgM and IgG2, what justifies the determination of a possible relationship between the presence of these IgM antibodies

and the development of preeclampsia. However, perhaps not only the presence of this antibody, but the levels of reactivity may be suggested as possible markers.

The objective of this study was to determine the levels of anticardiolipin antibodies in serum samples of pregnant women diagnosed with preeclampsia, comparing them with those of the control group to verify the possible association between the presence of these antibodies and the development of the syndrome.

For the conduction of this study, in the authorized period for sample collection, between March and August 2009, 55 pregnant women were studied, who were treated at the high-risk gynecology outpatient department of Hospital Universitário de Santa Maria (HUSM). This study assessed the presence of anticardiolipin antibodies in pregnant women with a diagnosis of preeclampsia ($n = 36$) and in healthy pregnant women ($n = 19$). All the studied patients were more than 20 weeks pregnant, once the criteria for the diagnosis of preeclampsia is gestational age ≥ 20 weeks.

IgM anticardiolipin antibodies were determined by immunoenzymatic assay (Elisa), using the commercial reagent Binding Site® (Bind Azyme™ Human Cardiolipin IgM Enzyme Immunoassay kit) and plate washer TP Washer NM and reader model TP Reader-NM of the brand Thermo Plate (São Paulo, Brazil) according to instructions by the manufacturer. The cut-off point of the reaction was standardized by the manufacturer; samples whose calculated reactivity value was ≥ 10 IgM phospholipid units (MPU)/ml were considered positive. The determination of anticardiolipin antibodies by the Elisa method has been standardized since the first symposium on APA, in 1987[12]. This study was submitted to and approved by the Research Ethics Committee of Universidade Federal de Santa Maria (UFSM) (CAAEE 0134.0.243.000-08). Total anonymity and privacy of patients were ensured, according to recommendations of Resolution nº 196/1996. All participants were duly informed about the study and signed the free informed consent form. For the statistical analysis, the unpaired t test was used to compare the continuous quantitative variables of normal distribution; Fischer's exact test and the chi-squared test with Yates' correction were used to assess qualitative parameters. Pearson correlation coefficient was used to determine the existence of association.

By means of the achieved results, we may verify a statistically significant difference among the groups, considering levels of systolic and diastolic BP, newborn weight ($p < 0.001$) and number of pregnancies ($p < 0.01$). With regard to patients' age, the number of miscarriages, family history of chronic hypertension and cases of preeclampsia or eclampsia in the family did not show a statistically significant difference ($p < 0.1$). Concerning gestational age, as expected, the average of both groups was similar: all patients had approximately 36.51 ± 0.4699 weeks of pregnancy, with no significant difference between the groups. The confirmed diagnosis of preeclampsia sometimes is made at the end of pregnancy, when all signs and symptoms are present. For this reason, pregnant women of the control group were selected in the same gestational period (**Table**).

Because urinary protein measurement is an important tool for the definition of preeclampsia, the results of 24-hour urine protein tests were associated with those for the detection of anticardiolipin antibodies. However, no statistically significant correlation between these parameters was observed ($r = 0.167$).

The presence of IgM anticardiolipin antibodies was detected in 13.89% of pregnant women with preeclampsia, when samples presenting values over 10 MPU/ml were considered positive. The reactivity level among pregnant women with diagnosis of

TABLE – Clinical characteristics of the studied pregnant women and relevant factors related to preeclampsia. Results are expressed in mean ± SD

	Control ($n = 19$)	Preeclampsia ($n = 36$)
Age (years)	26.63 ± 6.01	28.28 ± 7.72
Gestational age (weeks)	37.63 ± 0.5733	36.51 ± 0.4699
Diastolic pressure (mmHg)	74.21 ± 2.205	94.24 ± 1.89***
Systolic pressure (mmHg)	117.9 ± 2.712	150.2 ± 3.635***
Number of pregnancies	2.579 ± 0.4347	1.971 ± 0.1763**
Number of miscarriages	0.2632 ± 0.1683	0,2571 ± 0.08543
Number of nulliparous women	6 (31.58%)	18 (50%)
Number of pregnant women with history of preeclampsia in previous pregnancies	0	8 (33.33%)
Newborn weight (grams)	3,483 ± 101.6	2,636 ± 136.3***
Family history of chronic hypertension, gestational hypertension, preeclampsia or eclampsia	3	10

**p < 0.01; ***p < 0.001: statistically significant difference when compared with the control group.
SD: standard deviation.

preeclampsia was significantly higher than that found in the control group. The mean reactivity (MPU/ml) in the control group was 3.47, with standard deviation 2.17; in the group with preeclampsia it was 6.009, with standard deviation 3.95, being statistically different ($p < 0.05$).

We must highlight that it is not clear whether the increase in IgM anticardiolipin antibodies is the cause or the consequence of preeclampsia. While the literature data diverge about the influence of anticardiolipin antibodies in the development of preeclampsia, few studies have associated the search for IgM fraction with the pathology. Even so, recent studies have suggested that the presence of this class of antibody is one of the causes for the onset of preeclampsia, representing a risk factor for the disorder[13].

Anticardiolipin antibodies, once bound to phospholipids of the endothelial cell membrane, compete with the coagulation cascade activation, decreasing the contact area for the binding of vitamin K-dependent coagulation factors, thus producing a paradoxical anticoagulant effect. The procoagulant effect comes from the antibodies with lupus anticoagulant activity, which bind to phospholipids of the endothelial cell membrane, inhibiting G protein activation. This causes inhibition of the second messenger (cyclic adenosine monophosphate [cAMP]) production by G protein, directly affecting the synthesis of prostacyclin, the most potent natural known inhibitor of platelet aggregation. Reduced levels of prostacyclins, secreted by endothelial cells, will permit platelet aggregation, with the resulting thrombus formation. Hence, one may infer the influence of APA in the development of preeclampsia, since the unbalanced prostaglandin mechanism, represented by reduced prostacyclin activity, plays an important role in the development of preeclampsia[14].

If on one hand immunoassays are the standard technique for the detection of APA, on the other hand they are prone to interference, so it is necessary to be aware of their limitations. Antiphospholipid assays are affected by immune-mediated interferences (such as heterophile or human anti-animal antibodies, rheumatoid factor, high immunoglobulin levels), as well as by anticoagulants and the presence of residual platelets in the plasma. Despite the advanced knowledge on mechanisms of interference in antiphospholipid assays, it is unlikely that total elimination will be possible[15]. Moreover, the technical laboratorial factors of influence, genetic factors – such as family history –, and environmental factors – such as temperature variations – may also be associated with the development of preeclampsia and may have influenced the result[16].

Our results suggest the participation of anticardiolipin antibodies in the genesis of preeclampsia, what makes them possible markers for the disorder. Future studies in varied populations may confirm this hypothesis.

RESUMO

O objetivo deste estudo foi medir o nível de anticorpos anticardiolipina em gestantes pré-eclâmpticas a fim de verificar possível associação entre a presença desses anticorpos e o desenvolvimento desta síndrome. Foram avaliadas 36 gestantes com pré-eclâmpsia e 19 gestantes saudáveis. Os anticorpos anticardiolipina foram determinados por meio de ensaio imunoenzimático. O nível de reatividade dos anticorpos anticardiolipina foi significativamente mais elevado no grupo de pré-eclâmpticas, com nível de significância de 95%. Os resultados podem significar a participação dos anticorpos anticardiolipina na gênese da pré-eclâmpsia, e estudos futuros podem confirmar este parâmetro como marcador precoce do desenvolvimento dessa enfermidade.

Unitermos: pré-eclâmpsia; antifosfolípides; anticardiolipina; gestação.

REFERENCES

1. Moodley J. Maternal deaths due to hypertensive disorders in pregnancy. Best Pract Res Clin Obstet Gynaecol. 2008; 22(3): 559-67.

2. Cnossen JS, van der Post JA, Mol BWJ, et al. Prediction of pre-eclampsia: a protocol for systematic reviews of test accuracy. BMC Pregnancy Childbirth. 2006; 6: 29.

3. Ferraz L, Bordignon M. Mortalidade materna no Brasil: uma realidade que precisa melhorar. Rev Baiana Saúde Pública. 2012; 36(2): 527-38.

4. Villar J, Say L, Shennan A, et al. Methodological and technical issues related the diagnosis, screening, prevention, and treatment of pre eclampsia and eclampsia. Int J Gynaecol Obstet. 2004; 85 Suppl 1: S28-41.

5. Yamada H, Atsumi T, Kobashi G, et al. Antiphospholipid antibodies increase the risk of pregnancy-induced hypertension and adverse pregnancy outcomes. J Reprod Immunol. 2009; 79(2): 188-95.

6. Lyell D, Lambert-Messerlian GM, Giudice LC. Prenatal screening, epidemiology, diagnosis, and management of preeclampsia. Clin Lab Med. 2003; 23(2): 412-42.

7. Sibai B, Romero R, Klebanoff MA, et al. Maternal plasma concentrations of the soluble tumor necrosis factor receptor 2 are increased prior to the diagnosis of preeclampsia. Am J Obstet Gynecol. 2009; 200(6): 631-8.

8. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. N Engl J Med. 2002; 346(10): 752-63.

9. Wilson WA, Guaravi AE, Piette JC. International classification criteria for antiphospholipid syndrome: synopsis of a post-conference workshop held at the Ninth International (Tours) aPL Symposium. Lupus. 2001; 10(7): 457-60.

10. Samarkos M, Davies KA, Gordon C, et al. IgG subclasses distribution of antibodies against β2-GP1 in patients with lupus erythematosus and primary antiphospholipid syndrome, and their clinical associations. Rheumatology. 2001; 40(9): 1026-32.

11. Sammaritano LR, Ng S, Sobel R, et al. Anticardiolipin IgG subclasses: association of IgG2 with arterial and/or venous thrombosis. Arthritis Rheum. 1997; 40(11): 1998-2006.

12. Harris EM, Hughes GR. Standardising the anti-cardiolipin antibody test. Lancet. 1987; 329(8527): 277.

13. Kestlerová A, Feyereisl J, Frisová V, et al. Immunological and biochemical markers in preeclampsia. J Reprod Immunol. 2012; 96(1-2): 90-4.

14. Aron AL, Gharavi AE, Shoenfeld Y. Mechanisms of action of antiphospholipid antibodies in the antiphospholipid syndrome. Int Arch Allergy Immunol. 1995; 106(1): 8-12.

15. Lakos G. Interferences in antiphospholipid antibody assays. Semin Thromb Hemost. 2012; 38(4): 353-9.

16. Melo B, Amorim M, Katz L, et al. Hypertension, pregnancy and weather: is a seasonality involved? Rev Assoc Med Bras. 2014; 60(2): 105-10.

MAILING ADDRESS

Karla Nunes Pereira
Universidade Federal de Santa Maria; Hospital Universitário de Santa Maria; Laboratório de Análises Clínicas; Avenida Roraima, 1000, prédio 22; Camobi; CEP: 97105-900; Santa Maria-RS, Brazil; Phone: +55 (55) 3220-8712; e-mail: karlanunespereira@yahoo.com.br.