INTRODUCTION

Hyperprolactinemia is a physiologic or pathologic condition that causes hypersecretion of prolactin (PRL) by lactotroph cells. It was thought to be present in 10%-25% women with secondary amenorrhea or oligomenorrhea, in approximately 30% of women with galactorrhea or infertility, and in 75% of those with both amenorrhea and galactorrhea.
In human serum, three main species of PRL have been identified including the monomeric PRL (molecular mass 23 kDa) being the predominant form, the big PRL (molecular mass 50-60 kDa) and the big-big or macroprolactin (molecular mass 150-170 kDa). Jackson et al first used the new term "macroprolactinemia" to describe a patient with marked hyperprolactinemia whose PRL mainly consisted of macroprolactin. In the great majority of cases, macroprolactin was composed of a complex formed by an immunoglobulin G (IgG) and a monomeric PRL. Furthermore, in rare cases with slightly elevated PRL levels, non-IgG-bound forms of macroprolactin including complexes with IgA or IgM, highly glycosylated monomeric PRL, covalent, or noncovalent aggregates of monomeric PRL, have also been demonstrated.

The gold standard technique for the diagnosis of macroprolactinemia is the gel filtration chromatography (GFC), which is accurate, reproducible but also expensive, time-consuming, and labor intensive. The polyethylene glycol (PEG) precipitation has been widely used as a screening method, with which a number of studies showed that the PEG-induced precipitation of macroprolactin in serum sample represented a simple, inexpensive, and reliable screening assay for hyperprolactinemia differentiation. This test enabled the correct diagnosis of macroprolactinemia in at least 80% of the cases.

Macroprolactinemia is mostly defined as a type of hyperprolactinemia where more than 60% of circulating PRL is made up of macroprolactin. The recovery of PRL of >60% after precipitation with PEG 6000 usually indicated that macroprolactin was not present in significant amounts. With the recovery rate of 40%-60%, macroprolactin might be present and the lower the recovery the less likely this was the case of true hyperprolactinemia; with the recovery rate of <40%, it was typically consistent with the presence of substantial quantities of macroprolactin.

As the native state of macroprolactin is confined to the intravascular space, macroprolactin was shown to have differing degrees of in vitro biologic activity in most studies and it was found that most macroprolactinemic patients were asymptomatic. Several studies have identified the anti-PRL autoantibodies in the sera of patients with macroprolactinemia, consistent with the fact that most of the macroprolactinemia cases possessed PRL-IgG complexes.

Macroprolactinemia was considered to be a common finding in endocrinological practice with relative high incidence rates in the hyperprolactinemia population. Despite of many reports about the prevalence, laboratory diagnosis, and clinical manifestations of macroprolactinemia, little is known about its causes and the nature of the antibodies associated with the PRL-IgG complexes. In the present study, we aimed to evaluate the prevalence of antinuclear antibodies (ANA) and antithyroid antibodies in the patients with suspected macroprolactinemia. Moreover, the IgG subclasses of the PRL-IgG complexes were investigated and compared between the patient groups with different post-PEG recovery rates.

### 2 | MATERIALS AND METHODS

#### 2.1 | Patients

From January to July in 2018, 40 061 female patients visiting the Endocrinology Department of the Beijing Obstetrics and Gynecology Hospital were tested for the serum PRL levels. The subjects (between 20 and 40 years old) with prolactin >30 ng/mL (the upper limit of the current prolactin reference interval used in the laboratory) were included in the present study, in combination of the following exclusion criteria: pregnancy and lactation, under certain medication such as anti-depressant drugs, anti-hypertensive drugs, anti-gastric acid drugs, and some medical conditions other than pituitary tumors causing PRL abnormalities such hypothroidism.

This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital. Two millilitre venous blood was collected from each of the recruited patients followed by centrifugation and serum separation.

#### 2.2 | Reagents and methods

The serum prolactin was determined by the Siemens Centaur XP Chemiluminescent Immunoassay platform (Siemens, Ireland) with the prolactin reagent kit (Siemens, Cat. No. 09505871, USA). The macroprolactin screening was performed by the polyethylene glycol (PEG) 6000 (Sigma-Aldrich, Cat. No. 8074911000, Germany) experiment as previously described. Briefly, 200 μL of the PRL-elevated serum was well mixed with 200 μL of 25% PEG, followed by centrifugation 1500 × g for 30 minutes. The supernatant was re-analyzed for PRL, and the PRL recovery was calculated with the following equation: (2 × PRL level following PEG treatment/PRL level before PEG treatment) × 100. The thyroid peroxidase antibody (aTPO) IgG (Siemens, Cat. No. 10492399, USA) were also measured by the Siemens Centaur XP instrument mentioned above. The assay for antinuclear antibodies (ANA) was performed by the ELISA method on the TECAN Freedom EVO lyzer® (Switzerland) platform, with the ANA detection kits obtained from AESKU Diagnostics (Cat. No. 3119, Germany). The ELISA experiments were performed according to the manufacturer’s instructions. Briefly, the diluted sera were incubated in 96-well microplates for 30 minutes at room temperature. After the washing step, the conjugate was incubated and washed again before adding the substrate to generate enzymatic colorimetric reactions. The concentration of target antibody was calculated based on its OD (at the wavelength of 450 nm) value compared with the standard curve.

#### 2.3 | Measurement of IgG subclasses of anti-PRL autoantibodies

To evaluate the subclasses of IgG bound to the serum PRL, an immunoprecipitation experiments were carried out. Briefly 100 μL...
of each serum sample was incubated with the prolactin monoclonal antibody (Thermofisher, MiP0202, USA) cross-linked agarose (Enriching, MAg25K/NHS kit, China) at 4°C overnight with continuous shaking. After washing three times with PBS, the bound anti-PRL antibody-PRL-IgG complexes were then eluted with 0.1 M sodium citrate (pH 3.0) and further assayed by Western blotting. The Western blotting was performed as previously described. The eluted complexes of interest were separated in the SDS-12% PAGE (Beijing Biotides Biotechnology, WB1103, China) and transferred onto the nitrocellulose membranes (Whatmann). The membranes were then probed with the anti-human IgG1 antibody (ThermoFisher, A10648, USA), anti-human IgG2 antibody (SouthernBiotech, 9070-01, USA), anti-human IgG3 antibody (SouthernBiotech, 9210-01), or anti-human IgG4 antibody (SouthernBiotech, 9200-01) separately, followed by incubation with the IRDye™ secondary antibodies (1:20 000). Along with the patient serum, 0.5 ug of pure IgG1 (SouthernBiotech, 0151L-01, USA), IgG2 (Bio-Rad, 5225-3004, USA), IgG3 (SouthernBiotech, 0153L-01, USA), or IgG4 (Sigma-Aldrich, I4764, Germany) was loaded in each SDS-PAGE. The protein bands were visualized on a LiCor Odyssey instrument (LI-COR Biosciences, USA). The intensities of protein bands (IgG1-IgG4) in Western blots were determined with the ImageJ software (National Institutes of Health, USA) and normalized against the pure IgG1-IgG4 proteins.

2.4 Statistical analysis

The statistical analyses were performed using the SPSS software version 21.0 (IBM, USA). The differences between groups were compared by nonparametric Mann-Whitney U test. Categorical variables were compared using the chi-square test with Yates’s correction. A P value of <.05 was considered as statistically significant.

3 RESULTS

3.1 Clinical presentations in the patients with suspected macroprolactinemia

Of the 40 061 women visiting the Endocrinology Department, totally 317 patients with elevated serum prolactin level and meeting the exclusion criteria were subsequently subjected to the PEG precipitation screening assay. As shown in Figure 1, with the PEG screening, only 13 subjects had a PRL recovery rate of <40% (Group 1), compared with the 40 subjects with a recovery rate of 40%-60% (Group 2). As expected, the majority of the enrolled patients (n = 264) showed a recovery rate of >60% (Group 3), indicating the group of “true hyperprolactinemia.” More interestingly, the percentage of the patients with the typical clinical presentations (including decreased libido, infertility, gynecomastia, decreased bone mass, and galactorrhea) of the hyperprolactinemia in Group 1 (23.0%) was significantly lower than that in Group 2 (67.5%) and Group 3 (80.7%). In other words, the relative amount of the macroprolactin was negatively associated with the prevalence of the classic symptoms of the true hyperprolactinemia (Table 1).

3.2 Associations between autoantibodies and PRL recovery rates

As autoimmunity has been related to the prevalence and pathogenesis of hyperprolactinemia, the following autoantibodies including ANA, aTPO, and aTG were tested for all the Group 1 and Group 2 patients that were suspected for macroprolactinemia with low PEG recovery rates (<60%) (Figure 1). For control purpose, a portion of randomly selected Group 3 patients were also tested for ANA (n = 98) and antithyroid autoantibodies (n = 10) (due to limited serum

FIGURE 1 Schematic diagram for patient recruitment and study design
accessibility). As summarized in Table 2, the higher incidence rates of ANA, aTPO, and aTg were associated with the greater PRL recovery rates post-PEG precipitation, although the differences between Group 1 (46.1% for ANA, 7.7% for aTPO or aTg) and Group 2 (70.0% for ANA, 12.5% for aTPO or aTg) were not statistically significant. Interestingly, with the comparison between Group 1 and Group 3 in Table 2, no statistical difference was found for the positive rates of ANA or antithyroid autoantibodies (aTPO and aTg), suggesting that non-PRL-specific autoantibodies did not significantly contribute to the PEG precipitation. For the ANA testing, with close positive rates of ANA or antithyroid autoantibodies (aTPO and aTg), suggesting that non-PRL-specific autoantibodies did not significantly contribute to the PEG precipitation. As the PRL antibodies used in the commercial kits have different antigen specificity and reactivity,40-42 the incidence rates of suspected macroprolactinemia were less likely to exhibit the classic symptoms of the hyperprolactinaemic syndrome,33 it is therefore important to distinguish such individuals from those with true hyperprolactinemia to avoid unnecessary biochemical and imaging investigations or even inappropriate medical treatment.5,34,35

### TABLE 2  Associations between autoantibody positivity and PEG recovery rates

| % of ANAa positivity | % of aTPOb or aTgc positivity |
|----------------------|------------------------------|
| Group 1 (n = 13)     | 46.1% (6/13)                 |
| Group 2 (n = 40)     | 70.0% (28/40)                |
| Group 3 (n = 98)     | 41.8% (41/98)                |
| P value (Group 1 vs 3)| .767                         |
| P value (Group 2 vs 3)| .003                         |
| P value (Group 1 vs 2)| .221                         |

aAntinuclear antibodies.
bThyroid peroxidase antibody.
cAntithyroglobulin antibody.
dP value calculated from chi-square test.

4. DISCUSSION

The study was designed to investigate the laboratory and clinical significance of the women suspected for macroprolactinemia due to decreased PRL recovery rates post-PEG precipitation. As the macroprolactinemic patients with significant amount of prolactin-IgG complexes are less likely to exhibit the classic symptoms of the hyperprolactinaemic syndrome,33 it is therefore important to distinguish such individuals from those with true hyperprolactinemia to avoid unnecessary biochemical and imaging investigations or even inappropriate medical treatment.5,34,35

With the PEG precipitation screening assay that was universally adopted by clinical laboratories, 4.1% (13/317) of the enrolled patients with elevated serum PRL had the recovery rates of <40%, and 12.6% (40/317) had the recovery rates of 40%-60% (Figure 1), which was close to other findings.33,36 Many previous reports have indicated that the post-PEG recovery rate of 40% was an acceptable cutoff for macroprolactinemia screening purpose.15-17,37-39 However, because the PRL antibodies used in the commercial kits have different antigen specificity and reactivity,40-42 the incidence rates of suspected macroprolactinemia were highly variable, between 15% and 35%.10,25,26,43,44

As a result, Chen et al re-evaluated the cutoff of the recovery rates for the PEG screening assay and found that 50% for the i2000sr (Abbott Laboratories) and 60% for the E170 (Roche Diagnostics) were
optimum thresholds that were further verified by the GFC method.\textsuperscript{40} In our study, with the platform of Siemens Centaur XP used for PRL measurement, the patients of Group 1 (<40% recovery) and Group 2 (40%-60% recovery) seemed to be two distinct populations with variable manifestations of classic hyperprolactinemia symptoms (Table 1). Therefore, whether a different post-PEG recovery rate cutoff other than 40% exits in our PRL testing system needs to be further verified in combination with the gold standard GFC method.

It has been shown that autoimmune disorders were accompanied by increased PRL levels.\textsuperscript{45,46} Pelkonen et al reported three hyperprolactinemia cases in a 12 euthyroid-patient cohort.\textsuperscript{47} In another study, PRL was found to be significantly elevated in the patients with Hashimoto’s thyroiditis which is introduced by autoantibodies targeting thyroid.\textsuperscript{48} Similarly, Kramer CK et al observed increased prevalence of antithyroid antibodies in the presence of genuine hyperprolactinemia or macroprolactinemia, evidencing the association of PRL increase and antithyroid autoimmunity.\textsuperscript{49}

The hyperprolactinemia has been reported in the patients with different autoimmune disorders, such as systemic lupus erythematosus (SLE), antiphospholipid syndrome, rheumatoid arthritis (RA),
and a spectrum of connective tissue diseases. The ANA are a group of autoantibodies that bind to contents of the cell nucleus, and the test is widely used as an indicator for most of the autoimmune disorders mentioned above. The lower prevalence of both ANA and antithyroid antibodies in Group 1 than Group 2 supported the idea that non-specific autoantibodies such as ANA or antithyroid antibodies could precipitate with PEG less efficiently than the PRL-specific antibody. Interestingly, in general IgG1 and IgG3 were more likely found in IgG3 were the predominant IgG species in both Groups 1 and 2. In a study with a smaller group of macroprolactinemia patients (n = 6) reported by Hattori et al, it was found that IgG4 was the major subclass as it was observed in five of the six patients included, suggesting chronic antigen stimulation in those patients. With the similar experimental strategy to trap and determine the IgG subclasses of the PRL-IgG complexes but larger population suspected for macroprolactinemia (13 patients in Group 1 and 40 patients in Group 2), we found that IgG1 and IgG3 were the predominant IgG species in both Groups 1 and 2. Interestingly, in general IgG1 and IgG3 were more likely found in nonorgan-specific autoimmune conditions such as SLE and RA. Therefore, the origin and the development of the anti-PRL autoantibodies might share some similarity with those identified in SLE and RA. In conclusion, a significant portion (53/317) of the patients with elevated PRL were suspected for macroprolactinemia with the PEG precipitation screening. The patients with post-PEG PRL recovery rates of < 40% (Group 1) and 40%-60% (Group 2) were likely to represent two distinct populations of different clinical presentations, although the PRL assay-specific post-PEG recovery cutoff needs to be further optimized in our testing system. Lastly, the IgG1 and IgG3 were the predominant subclasses in the PRL-IgG complex trapped by the immunoprecipitation method, suggesting their pathogenic significance in the development of anti-PRL autoantibodies.

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CONFLICT OF INTEREST
The authors declare no conflict of interest. The sponsor had no role in the design, execution, interpretation, or writing of the study.

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REFERENCES
1. Vallette-Kasic S, Morange-Ramos I, Selim A, et al. Macroprolactinemia revisited: a study on 106 patients. J Clin Endocrinol Metab. 2002;87(2):581-588.
2. Mancini T, Casanueva FF, Giustina A. Hyperprolactinemia and prolactinomas. Endocrinol Metab Clin North Am. 2008;37(1):67-99.viii.
3. Ciccarelli A, Dal AF, Beckers A. The epidemiology of prolactinomas. Pituitary. 2005;8(1):3-6.
4. Vilas L, Vilar CF, Lyra R, Freitas MDC. Macroprolactinemia presenting like a pituitary tumor. Am J Med. 1985;78(2):346-350.
5. Hattori N. Macroprolactinemia: a new cause of hyperprolactinemia. J Pharmocol Sci. 2003;92(3):171-177.
6. Shimatsu A, Hattori N. Macroprolactinemia: diagnostic, clinical, and pathogenic significance. Clin Dev Immunol. 2012;2012:167132.
7. Kasum M, Oreskovic S, Cehic E, Sunj M, Lila A, Eubovic E. Laboratory and clinical significance of macroprolactinemia in women with hyperprolactinemia. Taiwan J Obstet Gynecol. 2017;56(6):719-724.
8. Kasum M, Pasic-Baldani D, Stanic P, et al. Importance of macroprolactinemia in hyperprolactinemia. Eur J Obstet Gynecol Reprod Biol. 2014;183:28-32.
9. Hattori N, Adachi T, Ishihara T, Shimatsu A. The natural history of macroprolactinemia. Eur J Endocrinol. 2012;166(4):625-629.
10. Vilar L, Abucham J, Albuquerque JL, et al. Controversial issues in the management of hyperprolactinemia and prolactinomas - An overview by the Neuroendocrinology Department of the Brazilian Society of Endocrinology and Metabolism. Arch Endocrinol Metabol. 2018;62(2):236-263.
11. Vilar L, Fleseriu M, Bronstein MD. Challenges and pitfalls in the diagnosis of hyperprolactinemia. Arq Bras Endocrinol Metabol. 2014;58(1):9-22.
12. Vilar L, Fleseriu M, Bronstein MD. Challenges and pitfalls in the diagnosis of hyperprolactinemia. Arq Bras Endocrinol Metabol. 2014;58(1):9-22.
13. Hauache OM, Rocha AJ, Maia AC Jr, Maciel RM, Vieira JG. Screening for macroprolactinemia and pituitary imaging studies. Clin Endocrinol (Oxf). 2002;57(3):327-331.
14. Fahie Wilson MN, Soule SG. Macroprolactinaemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on precipitation with polyethylene glycol. Ann Clin Biochem. 1997;34(Pt 3):253-258.
15. Schlesche JA. The macroprolactin problem. J Clin Endocrinol Metab. 2002;87(12):5408-5409.
16. Fahie Wilson M. In hyperprolactinemia, testing for macroprolactin is essential. Clin Chem. 2003;49(9):1434-1436.
17. Glezer A, Soares CR, Vieira JG, et al. Human macroprolactin displays low biological activity via its homologous receptor in a new sensitive bioassay. J Clin Endocrinol Metab. 2006;91(3):1048-1055.
18. Hattori N, Ishihara T, Ikekubo K, Moridera K, Hino M, Kurahachi H. Autoantibody to human prolactin in patients with idiopathic hyperprolactinemia. J Clin Endocrinol Metab. 1992;75(5):1226-1229.
19. Hattori N, Inagaki C. Anti-prolactin (PRL) autoantibodies cause symptomatic hyperprolactinemia: bioassay and clearance studies of PRL-immunoglobulin G complex. J Clin Endocrinol Metab. 1997;82(9):3107-3110.
20. Oluokoga AO, Kane J. Anti-prolactin autoantibodies and hyperprolactinaemia. Eur J Endocrinol. 1995;133(4):463-464.
21. Cavaco B, Leite V, Santos MA, Arranhado E, Sobrinho LG. Some forms of big big prolactin behave as a complex of monomeric prolactin with an immunoglobulin G in patients with macroprolactinemia or prolactinoma. J Clin Endocrinol Metab. 1995;80(8):2342-2346.
22. Pascoe-Lira D, Duran-Reyes G, Contreras-Hernandez I, Manuel-Apolinar L, Blanco-Favela F, Leanos-Miranda A. Frequency of
macroprolactinaemia due to autoantibodies against prolactin in pregnant women. J Clin Endocrinol Metab. 2001;86(2):924-929.

24. De Schepper J, Schiettecatte J, Velkeniers B, et al. Clinical and biological characterization of macroprolactinaemia with and without prolactin-IgG complexes. Eur J Endocrinol. 2003;149(3):201-207.

25. Hattori N, Ishihara T, Saiki Y, Shimatsu A. Macroprolactinaemia in patients with hyperprolactinaemia: composition of macroprolactin and stability during long-term follow-up. Clin Endocrinol (Oxf). 2010;73(6):792-797.

26. Elenkova A, Genov N, Abadzhieva Z, et al. Macroprolactinaemia in patients with prolactinomas: prevalence and clinical significance. Exp Clin Endocrinol Diabetes. 2013;121(4):201-205.

27. Jackson RD, Wortsman J, Malarkey WB. Characterization of a large molecular weight prolactin in women with idiopathic hyperprolactinemia and normal menses. J Clin Endocrinol Metab. 1985;61(2):258-264.

28. Hattori N, Ikekubo K, Nakaya Y, Kitagawa K, Inagaki C. Immunoglobulin G1 and prolactin (PRL) isoforms in macroprolactinaemia due to anti-PRL autoantibodies. J Clin Endocrinol Metab. 2005;90(5):3036-3044.

29. Suliman AM, Smith TP, Gibney J, McKenna TJ. Frequent misdiagnosis and mismanagement of hyperprolactinemic patients before the introduction of macroprolactin screening: application of a new strict laboratory definition of macroprolactinaemia. Clin Chem. 2003;49(9):1504-1509.

30. Zhu H, Wang M, Dong Y, et al. Detection of non-criteria autoantibodies in women without apparent causes for pregnancy loss. J Clin Lab Anal. 2019;33(9):e22994.

31. Hu S, Sun H, Yin L, et al. PKR-dependent cytosolic cGAS foci are necessary for intracellular DNA sensing. Sci Signal. 2019;12(609):eaav7934.

32. Borba VV, Zandman-Goddard G, Shoenfeld Y. Prolactin and autoimmunity. Front Immunol. 2018;9:73.

33. Jamaluddin FA, Sthaneshwar P, Husain O, Othman N, Chan SP. Importance of screening for macroprolactin in all hyperprolactinaemic sera. Malays J Pathol. 2013;35(1):59-63.

34. Olukoga AO, Kane JW. Macroprolactinaemia: validation and application of the polyethylene glycol precipitation test and clinical characterization of the condition. Clin Endocrinol (Oxf). 1999;51(1):119-126.

35. Heaney AP, Laing I, Walton L, Seif MW, Beardwell CG, Davis JR. Misleading macroprolactinaemia in pregnancy. Lancet. 1999;353(9154):720.

36. Jassam NF, Paterson A, Lipiatti C, Barth JH. Macroprolactin on the Advia Centaur: experience with 409 patients over a three-year period. Ann Clin Biochem. 2009;46( Pt 6):501-504.

37. Fahie-Wilson MN, John R, Ellis AR. Macroprolactin; high molecular mass forms of circulating prolactin. Ann Clin Biochem. 2005;42(Pt 3):175-192.

38. Gibney J, Smith TP, McKenna TJ. Clinical relevance of macroprolactin. Clin Endocrinol (Oxf). 2005;62(6):633-643.

39. Fahie-Wilson MN. Polyethylene glycol precipitation as a screening method for macroprolactinaemia. Clin Chem. 1999;45(3):436-437.

40. Chen YJ, Song GZ, Wang ZN. A new criteria for screening macroprolactinaemia using polyethylene glycol treatment combined with different assays for prolactin. Eur Rev Med Pharmacol Sci. 2016;20(9):1788-1794.

41. Smith TP, Suliman AM, Fahie-Wilson MN, McKenna TJ. Gross variability in the detection of prolactin in sera containing big big prolactin (macroprolactin) by commercial immunoassays. J Clin Endocrinol Metab. 2002;87(12):5410-5415.

42. Fahie-Wilson M, Biegelmayer C, Kratzsch J, et al. Roche Elecsys Prolactin II assay: reactivity with macroprolactin compared with eight commercial assays for prolactin and determination of monomeric prolactin by precipitation with polyethylene glycol. Clinical laboratory. 2007;53(7-8):485-492.

43. Vilar L, Naves LA, Freitas MC, et al. Clinical and laboratory features greatly overlap in patients with macroprolactinaemia or monomeric hyperprolactinaemia. Minerva Endocrinol. 2007;32(2):79-86.

44. Bjoro T, Morkrid L, Wergeland R, et al. Frequency of hyperprolactinemia due to large molecular weight prolactin (150-170 kD PRL). Scand J Clin Lab Invest. 1995;55(2):139-147.

45. Lavalle C, Loyo E, Paniagua R, et al. Correlation study between prolactin and androgens in male patients with systemic lupus erythematosus. J Rheumatol. 1987;14(2):268-272.

46. Mateo L, Nolla JM, Bonnin MR, Navarro MA, Roig-Escotet D. High serum prolactin levels in men with rheumatoid arthritis. J Rheumatol. 1998;25(11):2077-2082.

47. Pelkonen R, Salmi J, Lamberg BA. Interrelationship between TSH and prolactin secretion in patients with prolactinoma and autoimmune thyroiditis. Acta Endocrinol (Copenh). 1982;100(2):184-188.

48. Legakis I, Petroyianni V, Saramantis A, Tolis G. Elevated prolactin to cortisol ratio and polyclonal autoimmune activation in Hashimoto’s thyroiditis. Horm Metab Res. 2001;33(10):585-589.

49. Kramer CK, Tourinho TF, de Castro WP, da Costa OM. Association between systemic lupus erythematosus, rheumatoid arthritis, hyperprolactinaemia and thyroid autoantibodies. Arch Med Res. 2005;36(1):54-58.

50. Maran R, Dueymes M, Le Corre R, Renaudineau Y, Shoenfeld Y, Youinou P. IgG subclasses of human autoantibodies. Ann Med Interne. 1997;148(1):29-38.