Research Article

Prolactin May Not Play a Role in Primary Antiphospholipid (Hughes’) Syndrome

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1. Introduction

Prolactin (PRL) is a peptide hormone secreted from the anterior pituitary gland and regulated by tonic inhibition of the hypothalamus via dopamine [1]. It is secreted not only by the anterior pituitary gland, but also by many extrapituitary sites, including immune cells [2]. Pituitary secretion of PRL is stimulated by suckling and stress [2]. The relationship between PRL and the immune system has been demonstrated in the last two decades and has opened new windows in the field of immunoendocrinology. However, there are scarce reports about PRL in primary antiphospholipid syndrome (pAPS). The objective of this study was to evaluate PRL levels in patients with pAPS compared to healthy controls and to investigate their possible clinical associations. Fifty-five pAPS patients according to Sapporo criteria were age- and sex-matched with 41 healthy subjects. Individuals with secondary causes of hyperprolactinemia (HPRL) were excluded; demographic, biometric, and clinical data, PRL levels, antiphospholipid antibodies, inflammatory markers, and other routine laboratory findings were analyzed. PRL levels were similar between pAPS and healthy controls (8.36 ± 7.02 versus 8.71 ± 6.73 ng/mL, P = .876). Nine percent of the pAPS patients and 12.1% of the control subjects presented HPRL (P = .740). Comparison between the pAPS patients with hyper- and normoprolactinemia revealed no significant differences related to anthropometrics, clinical manifestations, medications, smoking, and antiphospholipid antibodies (P > .05). This study showed that HPRL does not seem to play a role in clinical manifestations of the pAPS, differently from other autoimmune rheumatic diseases.

2. Methods

2.1. Patients. This comparative, descriptive, case-control study was conducted at the Rheumatology Division of the Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo.
All patients fulfilled the 1997 revised Sapporo criteria for the diagnosis of APS [6]. Anthropometric data, clinical manifestations, and laboratory results from 55 pAPS patients were collected from the patients’ medical charts and compared with sex-matched healthy controls. Exclusion criteria were presence of other autoimmune diseases, such as SLE, use of drugs that are known to affect levels of PRL (i.e., psychotropic drugs, thyroid hormones, glucocorticoids, and estrogens or contraceptives), and patients with secondary causes of HPRL, such as primary hypothyroidism, end-stage renal disease, or prolactinomas.

Comparative analyses were carried out between sex, age, and disease duration. Anthropometric measurements including weight (kg), height (cm), and body mass index (weight/height²) were also performed. The following clinical parameters were evaluated: venous thrombosis (documented deep vein thrombosis and/or pulmonary embolism), arterial thrombosis (at least one of the following: documented peripheral arterial thrombosis, stroke, transient ischemic attacks, or acute myocardial infarction), livedo reticularis, thrombocytopenia, recurrent spontaneous abortions, and in utero fetal loss. In addition to the laboratory assessment of serum PRL, all sera of the patients were screened also for anticardiolipin antibodies, lupus anticoagulant, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR).

2.2. Antiphospholipid Antibodies. IgG and IgM anticardiolipin antibodies (ACLs) were estimated at least twice using an enzyme-linked immunosorbent assay (ELISA) as previously described [7]. There was an interval of 12 weeks between each measurement. Briefly, 50 μL of cardiolipin (Sigma, St Louis, MO, USA) dissolved at 50 μg/mL in ethanol was used to sensitize polystyrene microtiter plates that were left to dry overnight at 4°C. Nonspecific binding sites were blocked using 30% heat-inactivated fetal calf serum in PBS (FCS/PBS) for one hour. Fifty microliters of each serum sample diluted 1:50 in FCS/PBS was added in duplicate to the plates, followed by alkaline phosphatase-conjugated goat antihuman IgG (Sigma, St Louis, MO, USA). The manufacturer’s cutoff values were used. For the diagnosis of the syndrome, values above 20 GPL or MPL were considered positive according to the criteria established by Sapporo [6]. Lupus anticoagulant was measured according to international guidelines using activated partial thromboplastin time (APTT-Diagnostica Stago, France) and diluted Russell’s viper venom time (dRVVT-Trinity Biotech, Wicklow, Ireland) [8].

2.3. Inflammatory Markers. CRP was detected by nephelometry and ESR by modified Westergren.

2.4. PRL Measurement. They were measured by fluoroimmunometric assay (AutoDELFIA Prolactin, PerkinElmer Life analytic Science, Turku, Finland). Reference values for women are 2.0 to 15.0 ng/mL and for men are 2.0 to 10.0 ng/mL.

2.5. Statistical Analysis. Results are presented as mean and standard deviations or median (range) for continuous variables and as a number (%) for categorical variables. Data were compared by t-tests or by the Mann-Whitney test for continuous variables to evaluate differences among patients with pAPS and controls and among pAPS patients with and without HPRL. For categorical variables, differences were assessed by a chi-square test or Fisher’s exact test. P values less than .05 were considered significant.

3. Results

Table 1 shows the demographic characteristics, anthropometric measures, PRL levels, and inflammatory markers in patients with primary antiphospholipid syndrome (pAPS) and controls.

| Variable                  | pAPS       | Controls   | P     |
|---------------------------|------------|------------|-------|
| Age, years                | 42.03 ± 11.63 | 37.37 ± 11.81 | .053  |
| Female gender, n (%)      | 47 (85.5)  | 35 (85.4)  | 1.00  |
| Caucasian race, n (%)     | 49 (89.1)  | 34 (82.9)  | .548  |
| Disease duration, months  | 93.13 ± 61.96 | —          | —     |
| Weight, kg                | 74.54 ± 19.99 | 63.45 ± 8.68 | .0009 |
| Height, cm                | 155.53 ± 33.32 | 154.09 ± 34.94 | .830  |
| BMI, kg/cm²               | 29.2 ± 7.32  | 28.9 ± 7.85  | .0065 |
| Waist, cm                 | 90.63 ± 16.74 | 81.83 ± 8.14  | .0022 |
| PRL levels, ng/mL         | 8.94 ± 7.02  | 8.71 ± 6.73  | .876  |
| HPRL, n (%)               | 5 (9.0)      | 5 (12.1)    | .740  |
| CRP, mg/L                 | 4.52 ± 4.67  | 2.15 ± 2.60  | .0063 |
| ESR, mm/1st hour          | 13.81 ± 13.33 | 5.92 ± 4.30  | .0006 |

Data are presented as means ± standard deviations or percentages; t-tests and chi-square tests were used.

Table 1: Demographic characteristics, anthropometric measures, PRL levels, and inflammatory markers in patients with primary antiphospholipid syndrome (pAPS) and controls.
Table 2: Comparison between primary antiphospholipid syndrome patients (pAPS) with hyperprolactin and normal PRL levels.

|                      | pAPS with HPRL | pAPS with Normoprolactinemia | P   |
|----------------------|----------------|-----------------------------|-----|
| Age (years)          | 34             | 42                          | .053|
| Female gender, n (%) | 5 (100)        | 42 (84)                     | 1.00|
| Caucasian race, n (%)| 3 (60)         | 46 (92)                     | .086|
| Disease duration, months | 106 (27–189) | 82 (1–224)                  | .279|
| Weight, kg           | 70 (57–90)     | 75 (47–156)                 | .237|
| Height, cm           | 161 (158–168)  | 162 (140–180)               | .215|
| BMI, kg/cm²          | 27 (22.3–31.9) | 26.75 (27.5–42.2)           | .328|
| Waist, cm            | 81 (76–110)    | 91 (65–157)                 | .294|
| Sedentarism, n (%)    | 5 (100)        | 29 (58)                     | .144|
| Current smoking, n (%)| 0              | 8 (16)                      | 1.00|
| Previous smoking, n (%)| 0              | 20 (40)                    | .147|
| Arterial event, n (%)| 3 (60)         | 34 (68)                     | 1.00|
| Venous event, n (%)   | 2 (40)         | 28 (56)                     | .649|
| Obstetric event, n (%)| 1 (20)        | 21 (42)                     | .638|
| Stroke, n (%)         | 2 (40)         | 22 (44)                     | 1.00|
| Sneddon syndrome, n (%)| 0              | 11 (22)                    | .571|
| Limb ischemia, n (%)  | 2 (40)         | 6 (12)                      | .149|
| Systemic arterial hypertension, n (%) | 1 (20) | 23 (46) | .373|
| Acute Myocardial Infarction, n (%) | 0 | 1 (2) | 1.00|
| Angina, n (%)         | 0              | 7 (14)                      | 1.00|
| Deep venous thrombosis, n (%) | 2 (40) | 23 (46) | 1.00|
| Pulmonary thromboembolism, n (%) | 1 (20) | 9 (18) | 1.00|
| Thrombocytopenia, n (%) | 0              | 12 (24)                    | .574|
| CRP, mg/L             | 0.87 (0.64–19) | 3.22 (0.3–17.1)             | .494|
| ESR, mm/1st hour      | 8.1 (2–32)     | 9 (2–58)                    | .287|
| Lupus anticoagulant, n (%) | 3 (60) | 34 (68) | 1.00|
| Anticardiolipin IgM, n (%) | 1 (20) | 9 (18) | 1.00|
| Anticardiolipin IgG, n (%) | 2 (40) | 18 (36) | 1.00|
| Warfarin use, n (%)   | 4 (80)         | 46 (92)                     | .391|
| Chloroquine use, n (%)| 3 (60)         | 23 (46)                     | .659|
| Statin use, n (%)     | 1 (20)         | 17 (34)                     | 1.00|
| Acetylsalicylic acid use, n (%) | 2 (40) | 29 (58) | .643|

Data are presented as means (range or percentages); Mann-Whitney and Fischer tests were used. HPRL was defined as PRL > 10 ng/mL for men and >15 ng/mL for women.

Comparison between the hyperprolactinemic pAPS patients with normoprolactinemic patients revealed no significant clinical differences.

PRL enhances immunoglobulin production [9], which may contribute to increased autoreactivity. A variety of autoantibodies were observed in patients with HPRL, including antibodies to PRL, endothelial cells, cardiolipin, β2 glycoprotein I (β2GPI), Ro and La [10]. Praprotnik et al. [5] observed that HPRL is not associated with increased thrombosis; however, HPRL was more common among patients who had lupus anticoagulant activity as well as with some of APS major manifestations such as obstetric complications and did not relate to thrombosis. On the other hand, our study did not find any statistically significant association

obstetric events, stroke, deep venous thrombosis, pulmonary thromboembolism, angina, limb ischemia, myocardial infarct, and thrombocytopenia), drug use (warfarin, acetylsalicylic acid, chloroquine, and statin), current or previous smoking, and diagnosis of systemic arterial hypertension (P > .05) (Table 2).

4. Discussion

This study demonstrated that pAPS patients presented similar PRL levels compared to control (8.94 ± 7.02 ng/mL and 8.71 ± 6.73 ng/mL, respectively). However, among the patients (55) five (9.0%) showed hyperprolactinemia but an equal number was also found among the control group (41).
between pAPS with HPRL and normoprolactinemia in relation the presence of lupus anticoagulant, antiphospholipid antibodies, and clinical manifestations as thrombosis ones. According to other studies, PRL levels do not interfere directly in the modulating platelet function which suggests that the prothrombotic effect of this hormone may involve other cells types [11]. It is worth to mention that our population was possibly more homogenous (Table 1) than the one used by Fraprotnik and that genetic background may influence the results.

Obstetric events are associated with HPRL and antiphospholipid antibodies [5, 12] with impaired endometrial differentiation before conception. This mechanism contributes to the high incidence of pregnancy complications in APS [12]. Expression of decidual markers such as PRL, tissue factor (TF), signal transducer, and activator of transcription 5 (Stat5), but not insulin-like growth factor-binding protein 1 (IGFBP-1), is significantly lower in samples obtained from aPL (+) patients when compared with aPL (−) group [12]. In other words, patients with recurrent pregnancy loss have distinct endometrial gene expression profiles depending on the presence or absence of circulating aPL antibodies. In our study, only (1/5) patients with HPRL had obstetric event (pregnancy loss in the first trimester), and no significant correlation was found when pAPS were compared with normoprolactinemia (P = .64). However, this finding may be explained by the relatively small number of patients in our study.

Another interesting point of this study is that a wide variety of APS clinical manifestations were analyzed, such as neurologica, haematological, cardiac, pulmonary, and thrombotics ones, as well serological antibodies of this syndrome and anti-inflammatory markers. In SLE patients, PRL may have an effect on autoantibody production. Thus, autoimmune rheumatic disorders can be accompanied by increased PRL levels. Previous studies have demonstrated that lupus is associated with this endocrine alteration. The prevalence of HPRL in patients with SLE in most of the series ranges from 13 to 35% [13–15]. Moreover, one study [16] evaluated whether antibodies to PRL play a role in SLE patients with associated HPRL. They studied 259 consecutive SLE patients and suggested that anti-PRL antibodies could be the cause of HPRL in a subset of SLE patients, especially those with particularly high serum PRL levels with a diagnosis of idiopathic HPRL. The patients with anti-PRL antibody had fewer clinical manifestations and less serological activity, indicating that the biological activity of PRL was attenuated by the autoantibody. A meta-analysis demonstrated a significant increase in PRL concentrations in SLE patients. PRL likely stimulates lupus disease activity. Serum PRL and disease activity have been positively associated, and abnormally high PRL levels during pregnancy in SLE also correlate with disease activity [17]. According to the aforementioned, the best evidence for the association between PRL and human disease exists for SLE [18]. Curiously, another study which evaluated the prevalence and clinical significance of HPRL in APS concluded that HPRL was negatively related to arthralgias, venous thrombosis, pulmonary microthrombosis, pulmonary hypertension in APS, and neurological manifestations in pAPS (P < .05) [5]. However, our study found no connection between clinical manifestations and HPRL.

Certain limitations of our study must be addressed. Serum PRL levels were only measured at a single time point in our study and, thus, discrete alterations in the 24-hour secretion pattern of the hormone may have been missed [19]. In addition, factors other than circadian rhythms, such as age [20], menstrual cycle [21], sleep [22], and acute stressors [23], could have influenced our results.

Finally, our study showed that HPRL does not seem to play a role in clinical manifestations of the primary antiphospholipid syndrome, making it different from other autoimmune rheumatic diseases as SLE.

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