CLINICAL STUDY

Serum pituitary antibodies in normal pregnancy and in patients with postpartum thyroiditis: a nested case–control study

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Abstract

Objectives: The aim of this study was to evaluate antipituitary antibody (APA) prevalence in a series of patients with postpartum thyroiditis (PPT) during pregnancy and in the postpartum.

Design: We conducted a nested case–control study on consecutive PPT and normal pregnant women at the Centre for Endocrine and Diabetes Sciences in Cardiff and at the Department of Endocrinology in Pisa.

Methods: We enrolled 30 women with PPT: 17 were hypothyroid (Hypo), 7 with hyperthyroidism (Hyper) and 6 with a transient hyperthyroidism followed by hypothyroidism (Biphasic). Twenty-one healthy pregnant women served as controls. APA (measured using indirect immunofluorescence), free thyroxine, free triiodothyronine, TSH, antithyroid autoantibodies, and thyroid ultrasound were performed during pregnancy and postpartum. The stored sera have been sent to Pisa, where serum APA, IGF1, and cortisol were measured.

Results: APA were found in 8 out of the 30 PPT patients (26.7%) and in one normal pregnancy (4.7%, \(P = 0.063\)). Three out of the seventeen Hypo with PPT (17.6%), three out of the seven Hyper PPT (42.8%), and two out of the six Biphasic PPT (33.3%) were positive for APA. APA prevalence was not significantly different in the PPT subgroups (\(P = 0.453\)). With one exception, APA all increased in the postpartum period (87.5%, \(P < 0.016\)). Basal serum IGF1 and cortisol were in the normal range with the exception of two patients with positive APA who presented low serum IGF1 levels (36 and 45 ng/ml).

Conclusions: APA are frequently present in the postpartum period in patients affected by PPT. Further studies are necessary to evaluate whether APA in PPT patients are associated with pituitary function impairment.

Introduction

Circulating antipituitary antibodies (APA) have been detected in up to 57% of autoimmune hypophysitis (AH) (1) and in other autoimmune conditions including autoimmune thyroid diseases (9–56% in Hashimoto’s thyroiditis and 7–64% in Graves’ disease) (2–9), Addison’s disease (10), type 1 diabetes mellitus (11) and in autoimmune polyglandular syndrome (APS) (9, 11–14). The discrepancy observed in different series is likely due to patient selection and the different methods used for APA determination (15, 16).

APA are considered a marker of pituitary autoimmunity but their clinical utility in diagnosing or predicting AH still needs clarification.

AH shows an evident association with pregnancy. In a comprehensive review of 379 cases of AH, Catureglio et al. showed that 120 out of the 210 women with AH were diagnosed during pregnancy or in the postpartum. Interestingly, the majority of patients presented symptoms of AH in the last months of pregnancy and in the first few months after delivery (17–19).

Postpartum thyroiditis (PPT) is defined as a transient or permanent thyroid dysfunction occurring in the first year after delivery and is based on an autoimmune inflammation of the thyroid. PPT classically runs a biphasic course: a thyrotoxic phase followed by a hypothyroid period. Permanent or transient hypo- or hyperthyroidism alone may also be the outcome (20, 21). The prevalence of PPT is 7.5% with a range of 1.1–16.7% (22). However, when only patients with thyroid peroxidase antibodies (TPOAb) are considered, the frequency of developing subsequent PPT in early pregnancy is much higher (30–52%) (23). Interestingly, in patients with type 1 diabetes mellitus, higher frequencies of PPT have been described (15%) (24).

The prevalence of pituitary antibodies during pregnancy, and whether it is affected by the concomitant presence of other autoimmune diseases (or vice versa), is unknown.
The aim of the present study was to evaluate the prevalence of APA in healthy subjects during the pregnancy/postpartum period and in subjects with PPT.

Subjects and methods

Subjects

The women enrolled in this study belong to a large cohort of patients followed to investigate the relationship between pregnancy and autoimmune thyroid diseases. The study was carried out in 30 women (mean (±s.d.) age, 29 ± 5 years, range 19–38 years) with PPT, who were referred to the Endocrinology Unit of the University of Cardiff. Seventeen women were hypothyroid (Hypo), seven women had isolated hyperthyroidism (Hyper) and six women had transient hyperthyroidism followed by hypothyroidism (Biphasic). Twenty-one healthy pregnant women without any humoral sign of autoimmunity served as controls (Control; Table 1).

PPT was defined by the presence of impaired thyroid function, TPOAb, thyroglobulin antibodies (TgAb), and hypoeochoic pattern in thyroid ultrasonography in pregnant women (20).

Study design

This is a case–control study nested in a cohort study (25): sera from PPT patients and healthy controls were acquired during the first, second, and third trimester of pregnancy and at 6–14 and 18–24 weeks after delivery and stored at the Center for Endocrine and Diabetes Sciences in Cardiff. Serum free thyroxine (FT4), free triiodothyronine (FT3), TSH, TgAb, and TPOAb were immediately measured by commercial kits in Cardiff. Subsequently, the stored sera have been sent to the Department of Endocrinology in Pisa, where APA, IGF1, and cortisol were measured. Thyroid ultrasound was performed on all patients in the first trimester of pregnancy and after delivery, using a real-time instrument with a 7.5 MHz linear transducer, as previously described (26). None of the patients were taking glucocorticoids or other immunosuppressive therapy.

Table 1 Overall prevalence of APA in 30 patients with postpartum thyroiditis (PPT) and in normal pregnancy.

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<tr>
<th>PPT patients</th>
<th>Normal pregnancy</th>
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APA, antipituitary antibody; Hyper, hyperthyroid PPT patients; Hypo, hypothyroid PPT patients; Biphasic, PPT patients characterized by a thyrotoxic phase followed by a hypothyroid period.

No patients had any clinical sign or symptom of other autoimmune diseases.

Informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee.

Assays

Serum FT4 and FT3 (Vitros Immunodiagnostic Products, Ortho-Clinical Diagnostics Inc., Rochester, NY, USA), TSH (Immulite 2000 third generation TSH, Diagnostic Products Corp., Los Angeles, CA, USA) were assayed by commercial kits in all patients and controls. Normal values are as follows: FT4, 7.0–17.0 pg/ml; FT3, 2.7–5.7 pg/ml; TSH, 0.4–3.4 μIU/ml. TPOAb and TgAb were measured by enzyme linked immunosorbent assay (27) standardized against NIBSC 66/387 antithyroid microsome serum (National Institute for Biological Standards and Control, Holly Hill, London, UK). Antibody activity was considered to be normal when levels were <19.4 kIU/l for TPOAb and >98 kIU/l for TgAb. The intra- and interassay variations for TPOAb were 4.9% (at a mean of 155 kIU/l) and 7.6% (at a mean of 149 kIU/l) respectively; values for TgAb were 4.8% (mean 1350 kIU/l) and 7.2% (mean 1387 kIU/l) respectively. Serum IGF1 (BioSource SM-C-RIA-CT Europe S.A., Nivelles, Belgium) and cortisol (Immunotech, Marseille, France) concentrations were measured using commercial kits. Normal values were as follows: IGF1, 90–492 μg/l (16–24 years); 71–290 μg/l (25–50 years); early morning cortisol, 85–260 μg/l.

APA were assayed by indirect immunofluorescence (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) as described previously (9). In brief, serum diluted in PBS–Tween 1:10 was first incubated for 30 min at room temperature on five-well slides containing cryostat sections of monkey pituitary. After proper washing, fluorescein-conjugated goat anti-human immunoglobulins (IgAGM) were added. After the second 30 min incubation, slides were washed again and read with a fluorescence microscope. The APA test was considered positive starting at dilution 1/10.

All sera were evaluated blindly by two investigators (L.M and L.L.M). Samples were considered positive when a diffuse immunofluorescence pattern, showing an intracytoplasmic staining, was observed in the majority of fields; an agreement was always achieved in all cases of conflicting opinion (seven samples, 3%) after further readings. In each assay a positive and negative serum control was included. Indirect immunofluorescence is a qualitative method; in order to quantify the degree of positivity, all positive samples were further diluted at 1:30 and 1:90.

Statistical analysis

Data were expressed as mean ± s.d. for quantitative variables. Comparison between PPT and the control group for positive APA tests was performed using the
Fisher’s exact test. Comparison of APA variations between the beginning of pregnancy and postpartum in positive APA was performed by the MacNemar’s test. Comparison among PPT subgroups was performed using $\chi^2$ test. Mann–Whitney $U$-test was used for quantitative variables. Exact $P$ values were considered. The $P$ values <0.05 were considered as statistically significant.

**Results**

APA were found in 8 out of the 30 PPT patients (26.7%) and in one of the normal pregnant women (4.7%, $P$ value = 0.063). Three out of the seventeen Hypo pregnant women with PPT (17.6%), three out of the seven Hyper PPT women (42.8%), and two out of the six Biphasic PPT women (33.3%) had APA (Table 1). APA prevalence was not significantly different in the PPT subgroups ($P$ = 0.453).

In one Hypo PPT patient APA test was already positive at the start time of the study and remained positive during pregnancy and the postpartum period. This case was a 23-year-old woman who was in her first pregnancy and did not receive glucocorticoids or other immunosuppressive therapies. APA became positive during the second and third trimester in one Hypo PPT patient. Six to fourteen weeks after the delivery APA were found in eight patients and one control (Fig. 1). APA increased in the postpartum period in all positive APA patients with one exception (87.5%, $P$ < 0.016): in six patients they became positive (five 1:10 and one 1:90), one from the titer 1:10 increased to 1:30 and finally, one control subject became positive with a titer 1:30. APA titer was 1:10 in the majority of positive patients. In particular, two PPT women had 1:30 and 1:90 titer after delivery (Fig. 1).

**Figure 1** Titer and course of pituitary antibodies in patients with *postpartum* thyroiditis (PPT) during pregnancy (subdivided in first, second, and third trimester) and after delivery (6–14 and 18–24 weeks). Black circles indicate PPT patients with hypothyroidism, while circles show PPT patients with hyperthyroidism, and triangles show PPT patients with biphasic course. Control subjects are indicated with squares.

Serum IGF1 concentrations in patients and controls were in the normal range (188 ± 86 ng/ml, range 100–407 ng/ml and 203 ± 73 ng/ml, range 105–354 ng/ml respectively), with the exception of two patients with positive APA, who presented low serum IGF1 levels: one was hypothyroid (36 ng/ml) and one hyperthyroid (45 ng/ml). Early morning serum cortisol concentrations were normal both in patients and controls (157 ± 63 µg/l, range 86–275 µg/l and 178 ± 46 µg/l, range 98–248 µg/l respectively).

Serum TPOAb (7628 ± 5327 mU/ml versus 5385 ± 3721 mU/ml, $P$ = 0.017) but not TgAb (931 ± 1011 mU/ml versus 724 ± 810 mU/ml, $P$ = 0.17) levels were higher in APA positive than in APA negative patients. Likewise, serum TPOAb (7429 ± 4772 mU/ml versus 4537 ± 3264 mU/ml, $P$ < 0.0001) and TgAb (1060 ± 980 mU/ml versus 485 ± 623 mU/ml, $P$ < 0.0001) levels were significantly higher in the *postpartum* period than during pregnancy (Fig. 2).

Thyroid ultrasound showed a hypoechoic pattern in the majority of PPT patients. In the whole group of PPT

**Figure 2** Correlations between serum TPO (gray bars) and Tg antibodies (white bars) and pituitary antibodies. (A) Antithyroid antibodies titer (mean ± s.d.) in patients with and without pituitary antibodies. (B) Antithyroid antibodies titer (mean ± s.d.) in patients during pregnancy and in the *postpartum* period.

* $P$ = 0.017, APA+ versus APA–; ** $P$ < 0.0001, *postpartum* versus pregnancy.
patients, thyroid ultrasound mean value was 12.9 ± 6.7 ml (range 4–32 ml) during pregnancy and 10.2 ± 5.5 ml (range 4–25 ml) in the postpartum. No control subjects had thyroid nodules or ultrasound patterns characteristic of autoimmune thyroiditis.

Discussion

This study shows that in patients with PPT and positive APA test pituitary antibodies increase in the postpartum period (P<0.016). In the control group of pregnant women without any sign of humoral autoimmunity, APA were present in one patient (4.7%) and, also in this case, appear in the postpartum (Fig. 1). Finally, APA have a higher prevalence in patients than in controls.

PPT is an exacerbation of an underlying autoimmune thyroiditis after an amelioration of the inflammation during pregnancy. The immunological rebound that follows pregnancy precipitates the clinical expression of autoimmune thyroiditis. In patients with other autoimmune disorders, the prevalence of PPT increases, or even triples in type 1 diabetes mellitus. Several studies demonstrated that the evolution between autoimmune and non-autoimmune disorders. In AH but also in normal subjects and in other endocrine autoimmune diseases (17). A pattern of pituitary blood flow, possibly yielding it more prone to modifications of the immune system (17).

AH has a temporal association with pregnancy that remains largely unexplained. Caturegli et al. reported that 120 out of 210 women (57%) presented AH during pregnancy or in postpartum. During pregnancy the pituitary gland increases its dimension and modifies the pattern of pituitary blood flow, possibly yielding it more prone to modifications of the immune system (17). A recent study proposed a novel pituitary antigen, a protein sequence shared among GH1, which is mainly expressed in the pituitary, and the somatotropin in the placenta. The immune reaction against placental epitopes could spread to the pituitary, causing AH and providing a further explanation for the striking temporal association between AH and pregnancy (1).

Another support to pregnancy induced-AH is the finding of pituitary autoimmunity in patients with Sheehan’s syndrome. If we consider the studies performed in Sheehan’s syndrome patients, the prevalence of APA is widely variable, possibly due to the different methods used (28, 29). APA, in fact, were found in 63% (30) and 35% (31) of patients with Sheehan’s syndrome when immunoblotting or immunofluorescence were used respectively. Serum autoantibodies represent the main diagnostic tool for diagnosis of autoimmune diseases. In the case of AH, however, serum APA cannot be considered a reliable serological marker due to their low specificity and sensitivity. Previous studies demonstrated APA not only in AH but also in normal subjects and in other endocrine autoimmune and non-autoimmune disorders.

Only two studies, to our knowledge, have evaluated serum APA in pregnant women. Takao et al. examined APA by immunoblot analysis on human pituitary tissue in 55 women 1 month before and 2 weeks after delivery: only 2 out of the 55 postpartum sera (3.6%) had reactivity to 22 kDa antigen human pituitary cytosolic protein (32). However, a second study, using a different detection method (ELISA), did not find APA in any of the 12 healthy pregnant women (33). In our population of 21 normal pregnant women, we found that serum APA were absent during pregnancy and appeared at a high titer in one patient in the postpartum (4.7%) suggesting a possible link between pituitary autoimmunity and pregnancy.

Scanty data are available on the association between AH and PPT: a case report of a 34-year-old woman with reversible ACTH deficiency was reported. The authors hypothesized a diagnosis of AH after excluding Sheehan’s syndrome or pituitary tumor (34).

In the present study we report that there is an association between serum APA and PPT, and serum APA tend to appear in the postpartum, both in normal and PPT women.

In our previous paper, we reported a high prevalence of serum APA in autoimmune thyroid diseases (11.4%), in particular, in Hashimoto’s thyroiditis (13%), and in APS (20%), whereas in non-autoimmune thyroid disorders and in normal subjects serum APA frequency was very low (0.6%). Moreover, we found a growth hormone deficiency in 18% of APA positive patients associated with magnetic resonance imaging (MRI) abnormalities (9). When we compared PPT patients with the population of our previous study we found a higher prevalence of APA in PPT patients than in those with autoimmune thyroid diseases (P=0.02) and control subjects (P<0.0001). These data suggest that thyroid autoimmunity and pregnancy may be synergistic in triggering pituitary autoimmunity.

Whether APA represents an autoimmune marker of pituitary deficiency is a matter of discussion. Basal hormone measurements may not be sufficient to detect pituitary impairment and may require dynamic tests. The two APA positive patients with low serum IGF1 concentrations are probably affected by GH deficiency. If an MRI had been possible, it may very well have confirmed, together with the previous evidence of GH deficiency, pituitary abnormalities. However, in this nested case–control study, a dynamic pituitary function study and MRI were impossible due to the state of pregnancy.

In conclusion, this study shows that serum APA are frequently found in the postpartum period in patients affected by PPT. Further studies are necessary to evaluate if pituitary autoimmunity in PPT patients is associated with pituitary function impairment.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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