

## CLINICAL STUDY

# Anti-hypothalamus and anti-pituitary antibodies may contribute to perpetuate the hypopituitarism in patients with Sheehan's syndrome

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## Abstract

**Objective:** While anti-pituitary antibodies (APAs) were detected in some patients with Sheehan's syndrome (SS) suggesting an autoimmune pituitary involvement in the development of their hypopituitarism, hypothalamic cell anti-hypothalamus antibodies (AHAs) have not been investigated so far.

**Design:** The aim of this study was to evaluate the presence of AHA and APA in SS patients to verify whether an autoimmune hypothalamic–pituitary process can contribute to their late hypopituitarism.

**Methods:** Twenty women with SS with a duration of disease ranging from 3 to 40 years (median 25.5 years) were enrolled into the study. Out of 20 patients, 12 (60%) had panhypopituitarism and the others had partial hypopituitarism well corrected with appropriate replacement therapy. None of them had clinical central diabetes insipidus. AHA and APA were investigated by immunofluorescence method in all patients. In addition, a four-layer immunofluorescence method was used to verify whether AHA immunostained vasopressin-secreting cells (AVP-c) or not.

**Results:** AHAs were found in 8 out of 20 (40%) and APAs in 7 out of 20 (35%) patients with titers ranging from 1:32 to 1:128 and 1:16 to 1:32 respectively; however, in none of these positive patients AHA immunostained vasopressin cells. None of controls resulted positive for both antibodies.

**Conclusions:** Patients with SS, even many years after the onset of SS, can show antibodies to pituitary and/or hypothalamic but not AVP-secreting cells. Antibodies to unknown hypothalamic cells (releasing factor-secreting cells) other than APAs suggest that an autoimmune process involving both the hypothalamus and pituitary gland may contribute to late pituitary dysfunction in SS patients.

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## Introduction

Sheehan's syndrome (SS) classically refers to *post partum* hypopituitarism due to pituitary necrosis occurring during severe hypotension or shock secondary to massive bleeding (1). The frequency of SS has gradually decreased in developed countries as a result of improved obstetrical care. Sheehan estimated the prevalence of the syndrome as 100–200 per 1 000 000 women nearly 40 years ago (2). However, recent data suggest that it is getting more frequent in western population also. SS was the sixth most frequent cause of GH deficiency among 1034 patients (3). It is still a serious health problem in developing countries and is characterized by varying degrees of anterior pituitary dysfunction (4–6).

The certain pathogenesis of SS is not clearly understood but the basic process is the infarction due to arrest

of blood flow to the anterior lobe of the pituitary gland, and it may be due to vasospasm, thrombosis, or vascular compression (7). It is commonly characterized by slow progression of pituitary dysfunction several years after the last labor suggesting other factors in the pathogenesis of the disease. The presence of anti-pituitary antibodies (APAs) has been demonstrated in some patients with SS suggesting that an autoimmune pituitary process could be involved in this syndrome (8–11). In particular, sequestered antigens due to tissue necrosis could trigger autoimmunity and may cause delayed hypopituitarism in these patients (11). However, other authors did not detect APAs in this syndrome (12).

Moreover, as far as we know the presence of antibodies against hypothalamic cells in patients with SS has not been investigated so far, despite the partial diabetes insipidus that can occur in some of them (13).

To identify whether an autoimmune process at hypothalamic–pituitary level could be responsible for the late hypopituitarism in some patients with SS, we planned this study with the aim of investigating the occurrence of anti-hypothalamus antibodies (AHAs) and APAs in a large group of SS patients.

## Patients and measurements

### Patients

Twenty women with SS, with an age ranging from 35 to 70 years (median 53 years), a last labor age ranging from 18 to 34 years (median 29.5 years), and a duration of disease ranging from 3 to 40 years (median 25.5 years), were enrolled into the study. None of them had clinical diabetes insipidus.

The diagnosis of SS had been made according to the following criteria (4–6): 1) history of *post partum* hemorrhage and/or history of *post partum* failure of lactation and/or secondary amenorrhea; 2) varying degrees of loss of pituitary hormone reserve; 3) good clinical response to hormonal replacement therapy; and 4) exclusion of pituitary mass lesion and presence of empty sella or partial empty sella on magnetic resonance imaging (MRI).

Patients with SS were enrolled at the Erciyes University Medical School Department of Endocrinology. All patients gave their informed consent to the study, which was approved by the Local Ethics Committee.

### Immunological evaluation

AHAs and APAs were evaluated in patients with SS and in 50 female age-matched normal controls with prior conception without history of *post partum* hemorrhage and with time span mean from the last labor, statistically not different from the patients with SS.

### Hypothalamic cell antibody detection

AHAs were detected by an indirect immunofluorescence method on cryostat section of young baboon hypothalamus supplied by Biosystem, Italia, SRL (San Martino, Buon Albergo VR, Italy) as previously described (14). In particular, fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulin class G (IgG) sera were used to detect the presence of AHA. We chose to characterize AHA because none of the patients presented with clinical diabetes insipidus, in order to confirm or exclude an autoimmune involvement of vasopressin-secreting cells. This characterization in sera AHA positive was performed by a four-layer double immunofluorescence technique as described in our previous papers (14, 15). In particular, the same cryostat section, in a first immunostaining step, was tested against patient's serum and then FITC goat anti-human immunoglobulin sera,

and in a second immunostaining step against rabbit sera anti-AVP followed by rhodamine goat sera anti-rabbit IgG. We considered hypothalamic cell antibody specimens with titer  $\geq 1:8$  to be positive.

### APA detection

APAs were investigated by an indirect immunofluorescence method on cryostat section of young baboon pituitary gland supplied by Biosystem as previously described (16). In particular, FITC-conjugated goat anti-human IgG sera were used to detect the presence of APA; APAs were considered positive starting at dilution of 1:8.

### Pituitary function

In all cases hormone deficiency was diagnosed on basal hormone levels and appropriate dynamic tests including insulin tolerance test, thyrotropin-releasing hormone, and gonadotropin-releasing hormone luteinizing hormone-releasing hormone stimulation test (data not shown).

### Statistical analysis

Data are expressed as median range, unless otherwise specified. Non-parametric analysis was used because of the non-Gaussian distribution of the data. Differences among the AHA and APA titers were evaluated by Mann–Whitney test. In addition, Spearman's correlation analysis was performed to determine whether significant correlations were present between chosen parameters. A value of  $P < 0.05$  was considered statistically significant.

## Results

Clinical characteristics of patients are illustrated in Table 1. As regards to anteropituitary function, all patients had growth hormone (GH) deficiency (100%), associated with thyroid-stimulating hormone and gonadotropin deficiency in 18 (90%), prolactin deficiency in 17 (85%), and adrenocorticotrophin (ACTH) deficiency in 16 (80%) of them.

As a result, 12 out of 20 patients had panhypopituitarism and the other 8 without complete hypopituitarism were defined as having partial hypopituitarism.

The behavior of AHA in all patients and in normal controls is depicted in Fig. 1. AHAs were found in 8 of 20 patients (40%) with titer ranging from 1/32 to 1/128. None of the normal controls resulted positive for these antibodies. Immunofluorescence pattern in all sera positive for AHA was characterized by an intracytoplasmic staining in many hypothalamic cells (Fig. 2).

The use of a four-layer immunofluorescence method in the second sandwich rabbit serum anti-vasopressin and oxytocin showed that the cells targeted by these antibodies were not AVP-secreting cells but other, at the moment unknown, hypothalamic cells. AHA-positive patients had a duration of disease ranging from 6 to 40 years.

**Table 1** Baseline characteristics of patients with Sheehan's syndrome.

Patients no.	Age (years)	Onset of the disease (years) <sup>a</sup>	Duration of the disease (years) <sup>b</sup>	Pituitary MRI	AHAs titer	APAs titer	Hypopituitarism
1	53	28	25	ES	Absent	1/16	Complete
2	35	25	10	PES	1/128	Absent	Partial (LH/FSH-GH-TSH-PRL)
3	55	28	27	ES	Absent	1/16	Complete
4	60	34	26	ES	Absent	Absent	Complete
5	64	30	34	PES	Absent	Absent	Partial (LH/FSH-ACTH-PRL-GH)
6	49	23	26	ES	Absent	1/16	Complete
7	48	34	14	ES	1/64	Absent	Complete
8	53	32	21	ES	1/32	Absent	Complete
9	68	33	35	ES	Absent	1/16	Partial (LH/FSH-TSH-ACTH-GH)
10	53	29	24	PES	Absent	Absent	Complete
11	38	32	6	PES	1/128	Absent	Partial (LH/FSH-TSH-PRL-GH)
12	65	29	36	ES	Absent	Absent	Partial (LH/FSH-TSH-ACTH-GH)
13	52	28	24	ES	Absent	Absent	Partial (LH/FSH-TSH-PRL-GH)
14	36	28	8	ES	1/128	1/32	Complete
15	55	37	18	ES	Absent	1/32	Complete
16	62	27	35	ES	1/64	1/16	Complete
17	46	18	28	PES	1/32	Absent	Complete
18	70	30	40	ES	1/32	Absent	Complete
19	60	33	27	PES	Absent	Absent	Partial (GH)
20	35	32	3	ES	Absent	Absent	Partial (TSH-ACTH-PRL-GH)
Range	(35–70)	(18–34)	(3–40)				
Median	53	29.5	25.5				

MRI, magnetic resonance imaging; ES, empty sella; PES, partial empty sella; AHAs, anti-hypothalamus antibodies; APAs, anti-pituitary antibodies.

<sup>a</sup>The age of the last labor.

<sup>b</sup>Time interval between the last labor and the current age (time of sera withdrawal).

The behavior of APA in patients and in normal controls is depicted in Fig. 1. APAs were found in 7 out of 20 patients (35%) with SS with titers raging from 1/16 to 1/32 but in none of controls. Immunofluorescence pattern in all APA-positive sera was characterized by an intracytoplasmatic staining in many pituitary cells. (Fig. 2) APA-positive patients had a duration of disease from 8 to 35 years.

Moreover, titers of AHA were significantly higher than those of APA in positive patients ( $P < 0.03$ ).

Finally, a positive correlation has been evidenced between AHA ( $r = 0.47$ ;  $P = 0.03$ ) and APA ( $r = 0.57$ ;  $P = 0.01$ ) and a number of pituitary axes affected; no relationship was observed between AHA and APA and duration of the diseases.

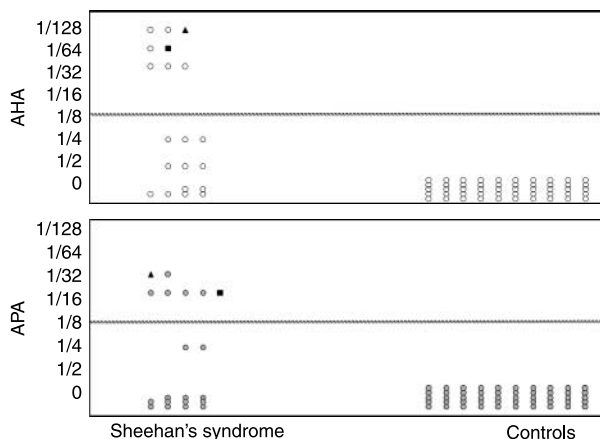
**Discussion**

This is the first study which has searched for AHA in patients with SS. In fact the role of the AHA in the hypothalamic–pituitary dysfunction in these patients has not been so far investigated. A recent study of the posterior pituitary function in SS patients without symptoms of central diabetes insipidus (CDI) demonstrated the occurrence of partial DI in 29.6% of them (13). However, the possible presence of AVP-secreting cells in these patients was not investigated.

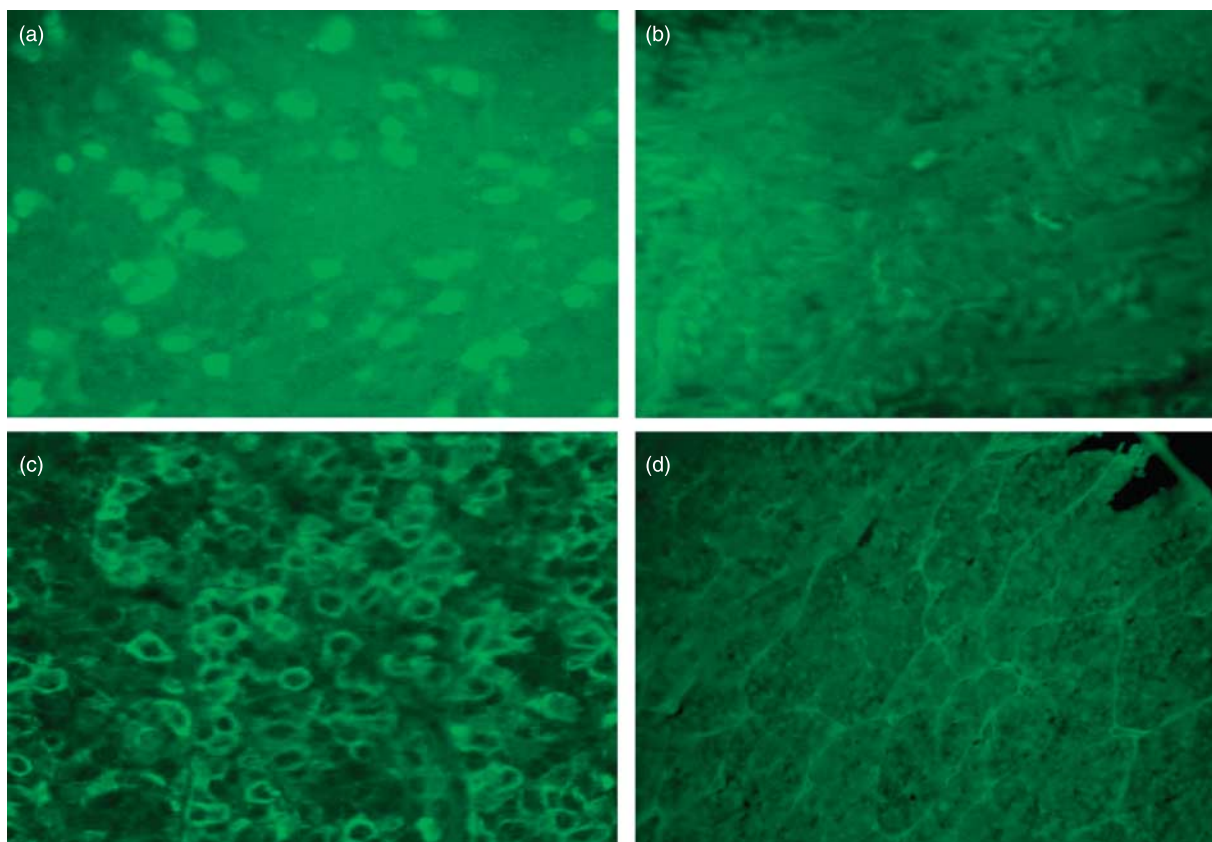
A very remarkable and surprising point emerging from our study is that, despite none of patients with SS here investigated having clinical diabetes insipidus, many of them (40%) resulted positive for antibodies against

hypothalamic neurosecretory cells, but the cells targeted by these antibodies were not vasopressin-secreting cells.

Previous longitudinal studies on a large cohort of patients with clinical autoimmune CDI (15) showed that AVPcAb in positive patients tend to decrease and/or disappear overtime. This could explain the unsuccessful detection of antibodies to AVP-secreting cells in our AHA-positive patients with SS. In fact, it cannot be excluded that some patients who resulted negative or positive for AHA at low titer ( $< 1:8$ ) in the present study (see Fig. 1) could have been significantly positive for



**Figure 1** Anti-hypothalamus antibodies (AHA; above) and anti-pituitary antibodies (APA; below) in patients with Sheehan's syndrome and in 50 female controls ▲ ■: the only two patients with high titers of both AHA and APA.



**Figure 2** Serum sample positive for antibodies to hypothalamic cells detected by immunofluorescence in an SS patient (a) in comparison with a negative control serum (b); serum sample positive for antibodies to pituitary cells detected by immunofluorescence in an SS patient (c) in comparison with a negative control serum (d).

AVPcAb in the previous years, taking into account the long-term mean duration of the disease.

Classical and conventional hypothalamic neurosecretory cells are not only neurons secreting arginine vasopressin or oxytocin but also hypophysiotropic neurons secreting their products (somatostatin, dopamine, and releasing factors) into the wells at the median eminence. However, while antibodies against AVP-secreting cells have been previously detected in patients with subclinical/clinical CDI (14, 15), the occurrence of antibodies against other hypothalamic neurosecretory cells in patients with hypopituitarism has not been so far investigated, except for an isolated finding of antibodies against the median-eminence dopaminergic nerve terminals in a patient with GH deficiency and autoimmune polyendocrine syndrome type 1 (17).

In the present study, we detected AHA not against immunostaining AVP-secreting cells but directed against other unknown secreting cells that could be putatively releasing factor-secreting cells. This could also explain the persistence of these antibodies at high titer in AHA-positive patients despite the long duration of the disease (until 40 years in one patient) in contrast with the behavior of AVPcAb, which, as previously said, tend to decrease or disappear overtime. Anyway, it can be suggested that an autoimmune process at

hypothalamic level could contribute to secondary pituitary impairment in some patients with SS. This seems to be also supported by the presence of pituitary function impairment in some positive only for AHA but not for APA.

Another point emerging from our study is that APAs are present in 35% of our patients with SS but in none of the normal females with prior conception and with normal obstetric history.

APAs, by indirect immunofluorescence, were detected in some patients with SS by some authors (8–10), but others could not demonstrate these antibodies in any patients with this syndrome (12). These conflicting results could be due to the difference in the pituitary sections used in screening for APA or to the small number of patients studied. To overcome these problems, Goswami *et al.* (11) used an immunoblotting method with pituitary homogenate as an alternative approach to detect APA in patients with SS. In particular, antibodies against a 49 kDa antigen were detected in 60% of patients with SS but also in 17.8% of normal females with prior conception. Moreover, the 49 kDa pituitary protein was identified as  $\alpha$ -enolase, an enzyme ubiquitously expressed; however, the importance of antibodies to enolase as markers of pituitary autoimmunity is still debated (18).

Our results confirm that pituitary autoimmunity may play a role in inducing late hypopituitarism following *post partum* hemorrhage.

Recent studies suggested that APA, when present at high titers, may be considered a good diagnostic marker of autoimmune pituitary involvement in patients with idiopathic hypopituitarism and in patients with autoimmune endocrine diseases (16, 19). In particular, we recently detected APA at high titers, targeting GH- (20) and gonadotropin-secreting (21) cells in some patients with idiopathic GH deficiency and in some cases of idiopathic hypogonadotropic hypogonadism, respectively, both isolated or associated with other pituitary dysfunctions. Interestingly, in our present study all patients showed GH deficiency and 18/20 (90%) of them showed hypogonadotropic hypogonadism associated with other pituitary hormone deficiencies. We suggest that a pituitary autoimmune process could play a role in causing late complex pituitary dysfunction in many females with SS. This seems to be also suggested by the finding of empty sella on MRI in all our patients, which could indicate at least in some of them the occurrence of a previous autoimmune hypophysitis (LYH) after the pituitary necrosis, taking into account that empty sella can be an unusual finding of LYH (22–24).

In conclusion, the detection of antibodies against hypothalamic but not vasopressin-secreting cells and pituitary cells suggests that an autoimmune process involving not only the pituitary gland but also the hypothalamus, probably triggered by sequestered autoantigens disclosed by the vascular alterations and the tissue necrosis, may be responsible for the late hypopituitarism in some patients with SS. Thus, to search for AHA in a large cohort of patients with hypopituitarism and the characterization of the hypothalamic cells targeted by these antibodies, which is in progress, could contribute to better clarifying this assumption.

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