

Prospective investigation of the hypothalamo–pituitary–adrenal axis in patients with tularemia

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Background/aim: To investigate prospectively the hypothalamo–pituitary–adrenal (HPA) axis by adrenocorticotrophic hormone (ACTH) stimulation test.

Materials and methods: Tularemia was diagnosed according to guidelines. An ACTH stimulation test (1 µg) and a dexamethasone suppression test (DST; 1 mg) were performed in patients in the acute phase of tularemia before antibiotic treatment and in the chronic phase.

Results: Nineteen patients (mean age: 41.0 ± 13.2 years; 57.9% female) with tularemia were enrolled in the study in 2011 and 2012. Cortisol response to ACTH stimulation test was sufficient in all patients during the acute phase. After the DST, the cortisol was not suppressed during the acute phase in only one patient. The median control time of 11 patients after acute tularemia was 13 months. During the chronic phase, cortisol response to ACTH stimulation was normal in all patients, and after DST cortisol was suppressed in all patients. The peak cortisol level after the ACTH stimulation test in the acute phase was higher than that in the chronic phase, but the difference was not statistically significant.

Conclusion: The HPA axis of patients with tularemia was not significantly affected in the acute and chronic phases.

Key words: Tularemia, adrenal insufficiency, hypothalamo–pituitary–adrenal axis

1. Introduction

Tularemia is a bacterial zoonotic diseases caused by *Francisella tularensis*. It exhibits different clinical manifestations depending on bacterial subspecies, such as *Francisella tularensis* (type A) or *holarctica* (type B), and the route of transmission, such as by arthropod bites, inhalation, or the ingestion of contaminated water or food. Tularemia caused by type B is much less severe than tularemia caused by type A, and fatal cases are rare in type B (1). In Turkey, the most common form of tularemia is oropharyngeal, caused by the subspecies *holarctica* (2,3).

The adrenal glands are affected in sepsis, brucellosis, and tuberculosis. Variations in adrenal response to tularemia are most prominent in volunteers with typical acute illness, and adrenal response is minimal or absent in those with mild symptoms (4,5). Sepsis-associated abnormal pituitary response may explain some factors, including anatomic damage, acute inflammation, and drug-related cell dysfunction (5). The hypothalamo–pituitary–adrenal (HPA) axis may become severely dysfunctional during sepsis (5–7). In the past, the adrenal

response of acute tularemia patients has been examined in healthy volunteers (8), but it is unclear whether there is a permanent adrenal insufficiency in the postinfectious period. The aim of this study was to investigate the HPA axis by adrenocorticotrophic hormone (ACTH) stimulation test in acute tularemia and after therapy in a prospective study.

2. Materials and methods

Adult tularemia patients who attended Erciyes University's Department of Infectious Diseases between 2011 and 2013 were included in this study. The study was approved by the local ethics committee, and informed consent was obtained from each patient. Tularemia was diagnosed in patients who had findings and symptoms compatible with tularemia, such as fever, sore throat, conjunctivitis, lymphadenopathy, cough and malaise, and tularemia microagglutination titer (MAT) of ≥1/128 or a 4-fold increase in titer after 4 weeks. Three of 6 clinical forms of tularemia were defined including oropharyngeal, oculoglandular, and glandular (1,2). Patients who were

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pregnant, under 16 years old, detected to have an abnormal rise in liver or renal function tests, or had a history of adrenal or pituitary diseases, and those using any drugs, including corticosteroid or antiepileptics, that have an effect on steroid metabolism, or oral contraceptives, were excluded from the study. Tularemia MAT was performed in the laboratory of the Public Health Agency of Turkey, in Ankara, which is a reference laboratory in Turkey. The ACTH stimulation test was performed before patients were given their first dose of antibiotics. Treatment for patients involved standard antimicrobial therapy such as streptomycin, gentamicin, ciprofloxacin, or doxycycline according to international guidelines (1). Failure was defined as persistence or recurrence of fever, re-enlargement of the lymph nodes, and ongoing constitutional symptoms with a rise in C-reactive protein (CRP) levels (9).

2.1. Evaluation of pituitary functions

On the first day of the study, basal hormone levels, including free triiodothyronine (T_3 ; normal range: 2.3–4.2 pg/mL), free thyroxine (T_4 ; normal range: 0.88–1.72 ng/dL), thyroid-stimulating hormone (TSH; normal range: 0.57–5.6 mIU/L), prolactin (normal range: 2.7–18.3 ng/mL), and ACTH (normal range: 10–50 pg/mL) were measured in the morning in all patients. A low-dose ACTH stimulation test was performed using 1 µg of ACTH (Synacthen, Novartis, Switzerland) intravenously before antibiotic administration, and cortisol levels were measured at 0, 30, and 60 min. A cortisol response to ACTH stimulation of >12.5 µg/dL was accepted as a sufficient response (10). Patients whose cortisol levels were found to be below the cut-off value in the low-dose ACTH test were defined as ACTH deficient. A low-dose dexamethasone suppression test (DST) was performed as follows: participants took a 1-mg tablet of dexamethasone at 2300 hours, and blood samples were obtained for measuring cortisol levels the next morning. The ACTH tests were performed at least 6 months apart.

We used the Kolmogorov–Smirnov test to compare normality of the variables. The paired samples t-test was used for numerical variables. Independent t-test was used to compare the means of two independent samples. $P < 0.05$ was considered statistically significant.

3. Results

Of the 19 patients enrolled in this study, the mean age was 41.0 ± 13.2 (range: 17–63) years, and 11 (57.9%) of the patients were female. The mean time interval between onset of symptoms and admission to hospital was 35.4 ± 27.4 (range: 3–120) days. Twelve (63.2%) of the patients had fever, and 18 (94.7%) patients presented with cervical mass, while another patient had tonsillitis. Clinical manifestations were enumerated as the oropharyngeal (14 patients, 73.7%), glandular (3 patients, 15.8%), and

oculoglandular (2 patients, 10.5%) forms. Other forms of tularemia, such as typhoidal and pulmonary, were not seen. Elevated erythrocyte sedimentation rate (ESR) (>20 mm/h) and CRP (>6 mg/L) were identified in 14 (73.6%) patients. Cortisol response to the low-dose ACTH stimulation test was sufficient in all patients. In only one patient, who had the oculoglandular form, was cortisol not suppressed during the acute phase (cortisol: 2.1 pg/mL) after DST. There was no statistical difference between patients with fever and without fever in terms of level of cortisol peak after ACTH stimulation (respectively, 22.9 and 22.5 pg/mL; $P = 0.879$). Likewise, there was no statistical difference between high levels of CRP and normal CRP in terms of level of cortisol peak after ACTH stimulation (respectively, 22.9 and 22.4 µg/mL; $P = 0.841$). In contrast, patients with normal CRP had statistically higher basal prolactin levels than those with high levels of CRP (respectively, 6.8 ± 2.5 and 19.5 ± 7.5 ng/mL; $P = 0.001$).

The median follow-up time of the 11 patients after acute tularemia was 13 months (range: 7 to 25). The mean basal and peak cortisol levels after stimulation with ACTH in the acute phase were higher than those in the chronic phase, but the difference was not statistically significant. The Table shows a comparison of cortisol levels and thyroid function test results in the acute and chronic phases. During the chronic phase, cortisol response to ACTH stimulation was normal in all patients, and cortisol suppression was achieved in all patients after DST. The patient who was unable to achieve cortisol suppression in the acute phase did not attend the follow-up test in the chronic phase.

4. Discussion

In the past, enlargement and involvement of the adrenal glands was detected in rat tularemia models (11). Experimentally, during human tularemia, the adrenal steroids of subjects were measured serially, and it was found that urinary extraction of adrenal steroids, namely 17-hydroxy corticosteroid, had increased. However, minimal or no adrenal response was detected in tularemia patients with moderate symptoms (8). Another experimental human study showed a decrease in free T_4 and an increase in 17-ketosteroid levels (12). This study is a clinical observation of adrenal response in humans. It was conducted in patients with glandular involvement including oropharyngeal, glandular, and oculoglandular cases. A low-dose ACTH stimulation test was used to evaluate adrenal response.

The HPA axis may be affected by some infections such as brucellosis, meningitis, and sepsis. Sepsis-associated abnormal pituitary response may occur as a result of anatomical damage and acute inflammation. The cytokines released in sepsis can also directly stimulate ACTH synthesis (5). In this study, the basal and peak cortisol

Table. Comparison of cortisol levels and thyroid function test in acute and chronic phases.

Variables	Acute phase (mean ± SD)	Chronic phase (mean ± SD)	P
Basal cortisol level (µg/dL)	12.95 ± 3.70	9.83 ± 3.06	0.253
TSH, mIU/L	1.43 ± 0.98	1.99 ± 1.08	0.168
Free T ₃ , (pg/mL)	2.84 ± 0.49	3.10 ± 0.43	0.153
Free T ₄ , (ng/dL)	1.33 ± 0.19	1.15 ± 0.25	0.098
Peak cortisol level* (µg/dL)	22.57 ± 5.47	19.29 ± 3.36	0.069

TSH: Thyroid-stimulating hormone, T₃: triiodothyronine, T₄: thyroxine.

*: With low-dose ACTH stimulation test.

levels of the patients in the acute phase were higher than those of patients in the chronic phase. However, there was no statistical difference between the cortisol levels in the acute and chronic phases. This may be explained by the fact that inflammation in the acute phase of tularemia was not as severe as other infections, such as acute brucellosis (4,10,13). One of the limitations of our study was that we could not include patients with severe forms of tularemia, such as pulmonary and typhoid forms, which have more potential to activate the HPA axis. On the other hand, there was no relation between peak cortisol level and high CRP level or fever in the acute phase of this study. It is known that CRP levels and fever are markers of acute inflammation. In this study, however, 14 patients had elevated CRP and ESR levels. Because patients were followed for at least 7 months from the onset of tularemia infection, CRP and ESR levels were normal in the chronic phase and were not compared with those in the acute phase. In a previous study on brucellosis, patients were examined after 6 weeks of therapy (4). Long follow-up periods after acute infection are important for the evaluation of pituitary function because acute inflammation may affect the HPA axis.

It has been reported that at 12 months after acute meningitis, growth hormone deficiency was detected in 42.8% of patients, and cortisol response to ACTH stimulation was insufficient in one patient. Furthermore, the antipituitary antibody was detected in 50% of patients (13). In our study, cortisol response to ACTH stimulation

was sufficient in all patients, both in the acute and chronic phases. Although it has been shown that inflammatory cytokines inhibit the release of the growth hormone-releasing hormone and stimulate prolactin release in sepsis (5), one study reported that of 14 patients with acute meningitis none had hyperprolactinemia in the acute phase (13). In this study, patients with a high CRP level had lower levels of basal prolactin than patients with normal CRP.

This study has some limitations, including the lack of measurement of adrenal size in both the acute and chronic phases. Because cortisol response to ACTH stimulation was normal, the importance of adrenal size measurement may decrease but should not be ignored.

In conclusion, the peak cortisol level induced by ACTH in the acute phase was higher than that in the chronic phase. However, the HPA axis of tularemia patients was not significantly affected in the acute and follow-up periods. This may be due to mild or moderate inflammation. Moreover, this inflammation may be related to lower prolactin level.

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