Investigation of the Hypothalamo-pituitary-adrenal Axis and Changes in the Size of Adrenal Glands in Acute Brucellosis

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Abstract. The aim of the study was to investigate the hypothalamo-pituitary-adrenal (HPA) axis by ACTH stimulation test and the changes in adrenal size in acute brucellosis before and after therapy in a prospective study. Sixteen patients with acute brucellosis and 15 healthy subjects were included in the study during the last two years. Cortisol levels were assessed before, 30 and 60 minutes after ACTH (250 µg i.v.) injection and the size of the adrenals was measured in both groups. Mean basal cortisol levels in the patients before the therapy and after the therapy were 22.1 ± 6.9 µg/dL and 11.3 ± 6.0 µg/dL, respectively. The difference was statistically significant (p<0.001). There was also statistically significant difference for basal cortisol levels between the healthy subjects (12.2 ± 4.6 µg/dL) and the patients before the therapy (p<0.001). Peak cortisol responses to ACTH were higher before the therapy in the patients with acute brucellosis (39.3 ± 10.7 µg/dL) than in the healthy subjects (30.4 ± 4.8 µg/dL, p = 0.014). However, there was no significant difference for peak cortisol levels in the patients before and after the therapy (32.7 ± 8.0 µg/dL). Mean basal cortisol levels and peak cortisol responses to ACTH between the patients after the therapy and the healthy controls were similar. Both the maximum width of the adrenal glands and the width of the adrenal limbs were significantly greater before the therapy compared to healthy subjects and post-treatment period. We concluded that the HPA axis is activated and the adrenal glands are enlarged in acute brucellosis, which is reduced after appropriate therapy.

Key words: Brucellosis, Adrenal function, ACTH test, Cortisol

BRUCELLA species are pathogenic bacteria of humans and animals, and are exceedingly well adapted to their hosts [1]. Human brucellosis is a potentially life-threatening multisystem disease, and is endemic in the Mediterranean region [2–5]. One of the early events in infection with intracellular bacteria is phagocytosis by resident macrophages and the release of cytokines by these cells. Cytokines, regarded as key players in brucellosis, are interleukin (IL)-12, IFN-γ, and tumor necrosis factor (TNF)-α. IL-12 is a key cytokine produced by B cells and macrophages and leads to Th1 immune responses in the host that will ultimately induce the secretion of interferon (IFN)-γ from T cells [4, 6, 7]. Several cytokines are known to affect the release of anterior pituitary hormones by an action on the hypothalamus and/or the pituitary gland. Cytokines (such as IL-1, IL-2, IL-6, IL-8, TNF-α) and IFN are chemical messengers that stimulate the HPA axis when the body is under stress or experiencing an infection [7, 8]. In our opinion, the adrenal glands may be also involved during acute brucellosis, but the exact frequency and extent of adrenal involvement in brucellosis are not well known.

The aim of this study was to investigate the HPA axis by ACTH stimulation test and the changes in adrenal size in acute brucellosis in a prospective study.
Materials and Methods

Patient population

Sixteen patients with acute brucellosis (9 females and 7 males; between 17 and 66 years of age, mean 42.8 ± 14.9 years) and 15 healthy subjects (12 females and 3 males; between 28 and 48 years of age, mean 36.5 ± 7 years) were studied prospectively. The patients with acute brucellosis and healthy subjects were comparable with regard to age and sex (p = 0.150 and p = 0.252, respectively). The patients were recruited from the Outpatient Clinic of Infectious Diseases, Erciyes University Hospital. Brucellosis was diagnosed based on the following criteria: (i) clinical history less than 8 weeks and findings compatible with brucellosis (fever, night sweats, arthralgia, hepatomegaly, splenomegaly and lymphadenopathy); (ii) positive blood culture and/or a significant standard tube agglutination test (TAT) of at least 1 : 160 and/or a fourfold or greater rise in titre following the onset of symptoms [1, 4]. Patients with another disease, pregnancy and any abnormalities in renal and liver function tests or using medicine including steroids known to affect the HPA axis were not included in the study. The patients were treated with doxycycline plus rifampicin or streptomycin for 6 weeks. All cases adjusted to the treatment and completely recovered clinically at the end of the treatment. Approval of the ethics committee was taken for the study, and in the control group subjects were informed about the study. The following data were recorded during trial period: complete blood counts, erythrocytes sedimentation rate, C-reactive protein (CRP). At least two blood cultures were taken and samples were cultured using the Bactec 9240 system (Becton Dickinson, USA) and standard tube agglutination test (TAT) were performed as described previously [9]. The tests were performed initially and at the end of the therapy.

Measurement of cortisol concentrations

ACTH stimulation test was performed with 250 µg of tetracosactrin (Synacthen, Ciba-Geigy, Wehr, Germany) given intravenously. The test was carried out at between 08.00 and 09.00 h in the patients with acute brucellosis and healthy subjects. Blood samples were taken immediately before the test and at 30 and 60 minutes afterwards. After centrifugation, plasma samples were stored at −20°C until assayed. Plasma cortisol concentrations were determined by radio-immunoassay (RIA) with a commercially available kit (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA). The intra- and inter-assay coefficients of variation were 5–7% and 6–7.9%, respectively. The cortisol response was defined as the difference between basal and peak cortisol concentrations. A plasma cortisol value of 20 µg/dL or more at any time during the test, including before injection, was taken as indicative of normal adrenal function [10, 11].

Radiological evaluation

CT scanning of the adrenals was carried out in 16 patients with acute brucellosis and 15 healthy subjects. All CT examinations were done with a third generation Toshiba XT scanner, using 2 mm section. CT examination was performed by the same investigator (ACD). CT scanning was done as previously reported: sufficient images were obtained to ensure that the entire gland was imaged. The maximum width of the gland (defined as the maximum width perpendicular to the long axis of the body of the gland, at the junction of the adrenal limbs and the body) and width of the adrenal limbs (defined as the maximum thickness of the medial and lateral limbs of the gland perpendicular to the long axis of the limb) were measured as previously described [12, 13]. Fig. 1 shows the technique of measurement of the adrenal gland size. ACTH stimulation test and CT scanning of the adrenals were repeated at the end of the therapy (on the 6th week) in the patients with acute brucellosis.

Statistical analysis

Statistical analysis was performed by using a paired and unpaired Student t, Fisher’s chi-squared and ANOVA (with Bonferroni) tests within-group comparisons, and between the patients and control groups. Values are expressed as mean ± SD. For all statistical analysis a p<0.05 was considered significant.

Results

Initial and post-treatment selected laboratory data of 16 patients with acute brucellosis are given in Table 1. Brucella species were isolated from the blood cultures.
in 12 cases (75%) and all strains were identified as *B. melitensis*. Both antibiotic regimens were well tolerated and the patients’ clinical condition improved remarkably in the sixth week of the treatment. At the end of the therapy, CRP and ESR significantly decreased and all positive blood cultures became routinely negative after the antibiotic therapy.

**Cortisol levels and cortisol responses to ACTH**

Mean basal cortisol levels were higher before the therapy (range 9.63–36.12 μg/dL) than after the therapy (range 6.21–21.54 μg/dL) in the patients with acute brucellosis, the difference was statistically significant (*p*<0.001, Table 2, Fig. 2). There was also significant

![Diagram of adrenal gland](image)

**Table 1.** Laboratory data of 16 patients with acute brucellosis

<table>
<thead>
<tr>
<th>Laboratory parameters</th>
<th>Before therapy</th>
<th>After therapy</th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte (/mm³)</td>
<td>6847.5 ± 3198.7</td>
<td>6806.3 ± 2610.6</td>
<td>0.944</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>45.2 ± 26.0</td>
<td>20.3 ± 13.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>39.9 ± 20.4</td>
<td>5.0 ± 3.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

![Plasma cortisol levels](image)

**Table 2.** Average values of basal and peak cortisol, cortisol responses and area under the curve (AUC) of cortisol levels in both groups

<table>
<thead>
<tr>
<th>Cortisol level (μg/dL)</th>
<th>Patient group</th>
<th>Control group</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before therapy</td>
<td>After therapy</td>
<td></td>
</tr>
<tr>
<td>Basal cortisol level</td>
<td>22.1 ± 6.9a</td>
<td>11.3 ± 6.0</td>
<td>12.2 ± 4.6</td>
</tr>
<tr>
<td>Peak cortisol level</td>
<td>39.3 ± 10.7b</td>
<td>32.7 ± 8</td>
<td>30.4 ± 4.8</td>
</tr>
<tr>
<td>Cortisol response</td>
<td>17.1 ± 8.1</td>
<td>21.2 ± 8.5</td>
<td>18.3 ± 5.9</td>
</tr>
<tr>
<td>AUC (μg × min/dL)</td>
<td>1870.8 ± 596.8c</td>
<td>1468.4 ± 358.1</td>
<td>1338.9 ± 216.2</td>
</tr>
</tbody>
</table>

*a* Significantly different than after the therapy group and control group (for each, *p*<0.001).

*b* Significantly different than control group (*p* = 0.014).

*c* Significantly different than after the therapy group (*p* = 0.03) and control group (*p* = 0.003).
difference for mean basal cortisol levels between the healthy subjects (range 8.08–21.79 μg/dL) and the patients before the therapy ($p<0.001$). Peak cortisol levels were higher before the therapy in the patients with acute brucellosis than in the healthy subjects ($p = 0.014$). Similarly, the area under curve of cortisol levels was higher in the patients before the therapy than after the therapy and control groups (Table 2, $p = 0.03$ and $p = 0.003$, respectively). However, there was no significant difference for peak cortisol levels in the patients before and after the therapy (32.7 ± 8.0 μg/dL). Mean basal cortisol and peak cortisol levels between the patients after the therapy and the healthy controls were similar. None of our 16 patients and 15 healthy subjects had adrenal insufficiency.

**Radiological findings**

Both the maximum width of the adrenal glands and the length of the adrenal limbs were significantly greater before the therapy compared to healthy subjects and post-treatment size of adrenal glands of the patients (Fig. 3). The findings are shown in Table 3.

**Discussion**

The HPA axis is activated during many bacterial and viral infections, resulting in an increase in circulating glucocorticoid levels. This can either occur centrally at the level of the hypothalamus or pituitary or through direct activation of the adrenal glands [14, 15]. HPA axis responses are increased during endotoxemia [14–16]. This activation of the HPA axis is probably the result of the action of inflammatory mediators such as cytokines [17,18]. Cytokines, regarded as key players in brucellosis, are IL-12, IFN-γ, and TNF-α. IL-12 is a key cytokine produced by B cells and macrophages [19]. The importance of cytokine responses in the pathogenesis of brucellosis has previously been studied in animal models [20]. In our opinion, the adrenal glands may also be involved during acute brucellosis due to release of inflammatory cytokines. However,

<table>
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<th>Table 3. Computerized tomography findings of patients and healthy subjects</th>
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<tbody>
<tr>
<td>Measurement (mm)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Maximum width</td>
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<tr>
<td>Right</td>
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<tr>
<td>Left</td>
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<tr>
<td>Width of lateral limb</td>
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<td>Right</td>
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<td>Left</td>
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<tr>
<td>Width of medial limb</td>
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<td>Right</td>
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<tr>
<td>Left</td>
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</tbody>
</table>

* Significantly different than after the therapy group and control group (for each, $p<0.001$).

^ Significantly different than after the therapy group and control group ($p = 0.015$ and $p = 0.001$, respectively).

^ Significantly different than after the therapy group and control group ($p = 0.003$ and $p = 0.014$, respectively).

^ Significantly different than after the therapy group and control group ($p = 0.009$ and $p = 0.003$, respectively).

^ Significantly different than after the therapy group and control group ($p = 0.009$ and $p = 0.001$, respectively).
the number of studies on the evaluation of the adrenal glands in human brucellosis is rather limited, and our study is the first attempt to investigate the HPA axis by ACTH stimulation test and the changes in adrenal size in acute brucellosis.

In this study, significantly higher levels of the mean basal cortisol were found in patients with acute brucellosis compared with end-treatment period or healthy subjects. Peak cortisol responses to ACTH were also higher before the therapy in the patients with acute brucellosis than in the healthy subjects. Since adrenal glands are maximally stimulated by endogenous ACTH seen in stressful conditions such as in acute brucellosis, plasma cortisol levels will be high and therefore exogenous ACTH may fail to further stimulate the adrenal glands to secrete cortisol [21]. Peak cortisol responses to ACTH stimulation have been detected at 60 minutes in most of the patients with acute brucellosis before the treatment. In contrast, most of the peak cortisol responses in the control group and in the patients with acute brucellosis after the treatment were measured at 30 minutes. This study shows that HPA axis is more activated in patients with acute brucellosis than healthy individuals and patients in remission. We can speculate that over-activated adrenals secrete more cortisol but may respond to ACTH more slowly, because the adrenals are strongly stimulated endogenously and the response to exogenous ACTH may be delayed.

It is known that, many bacterial and viral infections and bacterial proteins result in the activation of the HPA axis and increased glucocorticoid release [14, 15]. HIV infection may also result in activation of the HPA axis. Indeed, numerous studies have now shown increased serum cortisol levels in HIV-infected patients [22, 23]. The adrenal glands may be involved and enlarged by granulomatous infections (such as tuberculosis, histoplasmosis and blastomycosis), HIV and related infections [24–29]. Tuberculosis is still one of the most important infectious agents that causes adrenal involvement [27]. Kelestimur et al. [29] and Gulmez et al. [28] found that adrenal glands were enlarged during acute pulmonary tuberculosis, followed by a later decrease in the size of the adrenals at the end of the therapy, and they reported that adrenocorticoid function is increased.

We also investigated adrenal size by CT scanning in the patients before and after brucellosis therapy and in healthy subjects. Both the maximum width of the adrenal glands and width of the adrenal limbs were significantly greater before the therapy compared to healthy subjects and post-treatment size of adrenal glands of the patients. The adrenals are under stress in acute brucellosis and secrete more cortisol as a result of increased ACTH secretion by the pituitary gland. Therefore the adrenals become larger during active infection. An increase during acute brucellosis followed by a later decrease in the size of the adrenal glands after the 6-week brucellosis therapy were seen. There was no significant difference between the adrenal gland size in the healthy subjects and patients after brucellosis therapy.

In conclusion, adrenal glands are enlarged in patients with acute brucellosis, but return to normal size after the therapy. This study shows that the frequency of adrenal involvement demonstrated by CT scanning in patients with acute brucellosis is common. However, further research is needed to clarify the mechanisms of HPA activation in acute brucellosis and to determine whether increased cortisol levels are beneficial (anti-inflammatory) or harmful (immunosuppressive).

References

Effects of repeated injections of interleukin 1beta or lipopolysaccharide on the HPA axis in the newborn rat. *Cytokine* 11: 225–230.


