Adrenal Gland Irradiation Causes Fatigue Accompanied by Reactive Changes in Cortisol Levels

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Research

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Abstract

Background: Fatigue is a complicated syndrome associated with multiple factors. The development of fatigue after incidental radiotherapy (RT) to the adrenal gland has been observed in clinical practice. This study aimed to investigate the effect of adrenal RT on fatigue and related physiological impacts.

Methods: Three patients with inevitable RT to the adrenal gland were enrolled. Serum levels of cortisol, aldosterone, and adrenocorticotropic hormone (ACTH) were measured before, during, and after RT. Fatigue was scored according to the validated fatigue severity scale, and dosimetric parameters were collected. BALB/c mice were surgically explored for the identification of the left adrenal gland. Intra-operative RT was delivered with an electron beam. The swimming endurance test was applied for endurance assessment to represent fatigue. Plasma levels of stress hormones and histopathological features were examined.

Results: In the enrolled patients, serum baseline cortisol levels declined after RT. Alterations in levels of morning cortisol and aldosterone showed a similar trend to baseline cortisol, whereas ACTH levels increased, indicating possible compensatory feedback from the hypothalamic-pituitary-adrenal axis. In an experimental mouse model, RT to a unilateral adrenal gland decreased baseline cortisol levels and swimming endurance time. In the histopathological assessment, the irradiated adrenal glands showed characteristic RT injury features in the adrenal cortex.

Conclusions: The preliminary clinical observation of fatigue development and characteristic hormone alterations indicated that the adrenal glands could be regarded as an organ at risk from RT adverse effects. This observation was supported by functional and histopathological evidence from an experimental animal model.

Introduction

Fatigue is one of the most common adverse effects of cancer and cancer treatment [1, 2]. It is a complicated syndrome associated with multiple factors, such as progressive disease, anemia, pain, depression, sleep disturbances, poor nutrition, hormonal changes, and treatments including chemotherapy and radiotherapy (RT) [3–5]. The prevalence of fatigue in cancer patients, known as cancer-related fatigue, ranges from 25–99% in different reports [6]. After successful cancer treatment, fatigue remains problematic in 19% – 38% of patients [7].

Among the etiologies causing cancer-related fatigue, we observed a trend in clinical practice of the development of fatigue after incidental ionizing radiation to the adrenal gland. Fatigue may not resolve after rest and requires extended recovery [8]. Indeed, there are various clinical situations with incidental adrenal radiation in the era of dose-painting RT, such as RT for hepatocellular carcinoma (HCC), liver metastasis, portal vein thrombosis, vertebral metastasis, and para-aortic lymph node metastasis. However, the correlation between adrenal ionizing radiation (AIR) and fatigue remains unclear.
The adrenal glands consist of the outer connective tissue capsule, the cortex, and the medulla. The adrenal cortex and medulla have distinct embryologic and physiological functions [9]. The adrenal cortex is composed of three zones: the zona glomerulosa, zona fasciculata, and zona reticularis, which release mineralocorticoids, glucocorticoids, and androgens, respectively [10]. The zona fasciculata constitutes 75% of the adrenal cortex.

The essential glucocorticoids are cortisone and cortisol. Cortisone is the precursor of cortisol. Cortisol, the active form of glucocorticoids, has a half-life of approximately 3 hours, whereas cortisone has a half-life of only half an hour [11]. Cortisol is involved in increasing blood sugar levels, immune responses, protein synthesis, and the transformation of glucose into glycogen [12, 13]. Low levels of cortisol are related to fatigue due to its role in energy metabolism [14]. Cortisol is a stress hormone that is secreted in response to stress, such as fever, surgery, hypoglycemia, hypotension, and exercise [15, 16].

The hypothalamic–pituitary–adrenal (HPA) axis is a psychoneuroendocrine regulator of the stress response and the immune system. Adrenocorticotropic hormone (ACTH) stimulates the secretion of glucocorticoids from the adrenal cortex. ACTH is secreted from the anterior pituitary under the influence of corticotropin-releasing hormone (CRH) and arginine vasopressin. CRH secretion is regulated by circadian rhythms and additional stressors operating through the hypothalamus. The secretion of CRH and ACTH is inhibited by cortisol, highlighting the importance of negative feedback control [17]. ACTH and cortisol are secreted in a pulsatile fashion with a circadian rhythm, with levels highest upon awakening and then decline. Circadian rhythms are dependent on both day-night and sleep-wake patterns [18, 19]. In humans, cortisol levels peak in the morning with a nadir at night. Mice also display a circadian rhythm but peaking at night and with a nadir in the morning [20]. This study aimed to investigate the effect of adrenal RT on fatigue and related physiological impact in a human and experimental animal model.

**Methods And Materials**

**Human model**

**Patients**

Three patients with inevitable RT to the adrenal gland after standard optimization for intensity-modulated radiation therapy (IMRT) were prospectively enrolled. The patients were required to have a World Health Organization performance status of 0 to 2. All patients were treated with curative intent.

**Blood analysis**

Serum levels of cortisol at 08:00 and 16:00, aldosterone, and ACTH were measured on the day before RT, in the middle of the RT course, and 1 week after RT using radioimmunoassay kits. The cortisol levels at 16:00 were considered baseline cortisol levels according to the circadian rhythm.

**RT technique**
Patients underwent computed tomography (CT) simulation in the supine position and were immobilized with an alpha cradle. The adrenal glands were contoured while planning the CT images. The adrenal glands were located superior and anteromedial to the upper pole of the kidneys and appeared as a triangular or Y-shaped organ. IMRT was used for all patients. The treatment plans were generated using 6-MV - or 10-MV photons. All patients were treated with linear accelerators, and dosimetric parameters, including the mean dose of the left adrenal gland, were collected using RT planning systems (Eclipse Treatment Planning System v.13; Varian Medical Systems Inc., Palo Alto, CA, USA). Dose distributions for the planning and dose-volume histograms (DVHs) were recorded for evaluation (Fig. 1).

**Fatigue severity scale**

Fatigue was scored according to the validated fatigue severity scale (FSS), a short questionnaire for rating the levels of fatigue on the day before RT and 1 week after RT [21, 22]. The questionnaire contained nine statements that attempted to explore the severity of fatigue symptoms. Patients were required to circle a number from 1 to 7, depending on how appropriate they felt the statement applied to them during the past week. A low value indicated that the statement was not applicable, whereas a high value indicated agreement. The scoring was performed by calculating the average response to the questions. Patients with fatigue reported an averaged high value.

**Ethical statement**

This study was approved by the institutional review board of our institution with ethics committee approval and informed consent (IRB number: 20MMHIS132e).

**Experimental animal model**

**Experimental animals**

Four-week-old male BALB/c mice were purchased from the National Laboratory Animal Center, Taiwan. Mice were maintained at a temperature of 22 ± 1°C and humidity of 55 ± 10% with 12-hours of light (artificial illumination; 07:00-19:00). The mice were fed a commercial rodent diet (LabDiet 5001, PMI Nutrition International LLC, Brentwood, MO, USA) and allowed access to purified water in a water bottle.

After one week of pre-feeding, the mice were randomly grouped into four groups of four mice per group as follows: the sham group, the 0 Gy group, 0.5 Gy group, and 2 Gy group. The mice in the sham group only had fur shaved around the left flank area as controls. The mice in the 0 Gy group were surgically explored for the identification of the left adrenal gland after fur shaving. The mice in the 0.5 Gy group received a radiation dose of 0.5 Gy on the left adrenal gland after surgical exploration and fur shaving, whereas the mice in 2 Gy group received a dose of 2 Gy.

After grouping, the blood of all mice was collected at 15:00 and 20:00 for the measurement of plasma levels of stress hormones. The cortisol levels at 15:00 were considered the baseline cortisol levels according to the circadian rhythm. Surgery and radiation were arranged on the next day (Fig. 2a and 2b). The swimming endurance test was performed for exhaustion assessment to represent fatigue after
wound healing around 5 days after surgery and RT. Then another blood sampling was performed at 15:00 and 20:00 on the next day. Histopathology of the adrenal gland was sampled 7 days after surgery and RT.

All procedures were approved by the Experimental Animal Committee at MacKay Memorial Hospital (MMH-A-S-103-06), in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publication, 8th edition, 2011). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques.

**Blood analysis**

Blood samples were collected via retro-orbital blood sampling and detected using Drew Hemavet HV950 (Drew Scientific, Inc., Dallas, TX, USA). For serum biochemical parameters, blood samples were centrifuged (1000×g for 15 min at 4°C), and the serum was stored at −80°C until use. Serum cortisol levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits from Arbor Assays (Ann Arbor, MI, USA).

**RT technique**

After surgical exploration, intraoperative RT was administered to the left adrenal glands of the mice using a linear accelerator (IX, Varian, Palo Alto, CA) with an electron beam (6 MeV, 90%, dose rate: 400 MU/min).

**Exhausted swimming test**

The swimming ability of all mice was confirmed using the swimming test before the experiment. The swimming test was stratified according to studies performed by Matsumoto et al. An acrylic pool (90×45×45 cm) filled with water to a depth of 38 cm was used. The temperature of the water was maintained at 25 ± 0.5°C with a water heater and thermostat. The mice were allowed to swim until they failed to rise to the surface of the water to breathe within 7 s. The mice were rescued at the time to avoid drowning. For the swimming endurance test, all mice were loaded with lead wire weighted to 7% of body weight. These mice were then placed in a water tank for swimming (Fig. 2c). The duration of starting swimming to fail to rise within 7 s was recorded as the swimming endurance time [20, 23].

**Histopathology**

Formalin-fixed and paraffin-embedded adrenal glands from each group of mice were sectioned. Sections 4 μm thick were stained with hematoxylin and eosin (H&E).

**Statistical Analysis**

Statistical analysis was performed using SigmaPlot version 12.0 (Systat Software, Inc., CA, USA). Numerical data were expressed as mean ± standard deviation. A paired t-test was performed for FSS, serum levels of cortisol, aldosterone, and ACTH, which were measured before, during, and after RT for the human data and serum levels of cortisol in mice, which were measured before and after RT. The
swimming endurance time of different groups of mice was recorded, and the significance was analyzed using one-way ANOVA. Differences were considered significant if \( p < 0.05 \).

**Results**

**Human model**

**RT dosimetry**

In the enrolled patients, the average volume of the unilateral adrenal gland was \( 3.2 \pm 1.2 \text{ cm}^3 \). The mean RT dose to the unilateral adrenal gland was \( 33.5 \pm 11.8 \text{ Gy} \).

**Blood analysis**

The baseline serum cortisol levels declined from \( 10.4 \pm 3.9 \) (pre-RT) to \( 5.8 \pm 3.9 \) (interim RT) and \( 4.6 \pm 2.1 \) (post-RT 1 week) \( \mu \text{g/dL} \). The morning cortisol levels declined from \( 14.8 \pm 2.2 \) (pre-RT) to \( 9.4 \pm 3.5 \) (interim RT) and \( 10.2 \pm 1.8 \) (post-RT 1 week) \( \mu \text{g/dL} \) (Fig. 3a). The aldosterone levels declined from \( 11.5 \pm 3.3 \) to \( 8.5 \pm 3.7 \text{ ng/dL} \) (Fig. 3b). The ACTH levels increased from \( 86.5 \pm 59.4 \) to \( 98.9 \pm 59.8 \text{ pg/mL} \) (Fig. 3c). The baseline cortisol levels significantly declined after RT, as indicated by paired t-test analysis (\( p = 0.002 \)). Alterations in the levels of morning cortisol and aldosterone showed a similar trend to baseline cortisol. The ACTH levels increased after RT with a significant difference (\( p = 0.007 \)), indicating possible compensatory feedback from the HPA axis. Before RT, the difference between morning and baseline cortisol levels was insignificant (\( p = 0.62 \)) (Fig. 4a). However, there was a significant difference between morning and baseline cortisol levels after RT (\( p = 0.02 \)). It seemed that the circadian rhythm presented more prominent after RT (Fig. 4b).

**Fatigue severity scale**

The FSS score increased from \( 1.2 \pm 0.1 \) to \( 3.1 \pm 1.0 \) after RT (Fig. 5). This increase indicated that fatigue developed after RT.

**Experimental animal model**

**Blood analysis**

The baseline cortisol levels in the RT 2 Gy group significantly decreased from \( 274.6 \pm 65.4 \) to \( 193.6 \pm 50.9 \text{ ng/mL} \) (\( p = 0.007 \)), as indicated by paired t-test analysis (Fig. 6). The evening cortisol levels in the sham group significantly increased from \( 237.4 \pm 10.7 \) to \( 265.0 \pm 18.7 \text{ ng/mL} \) (\( p = 0.04 \)), whereas the levels in the 0.5 Gy and 2 Gy groups decreased from \( 376.5 \pm 4.9 \) to \( 239.7 \pm 50.4 \text{ ng/mL} \) (\( p = 0.05 \)) and \( 275.5 \pm 68.3 \) to \( 217.4 \pm 57.5 \text{ ng/mL} \) (\( p = 0.05 \)) with borderline significance (Fig. 7). The circadian rhythm was not apparent in all groups (Fig. 8) before or after the experiment. There was no significant difference in hemoglobin, white blood cell counts, or platelets among the different groups of mice.
**Swimming endurance time**

The swimming endurance time dose-dependently declined following RT, as 3.3 ± 0.2, 3.7 ± 0.4, 3.4 ± 0.0 and 1.7 ± 0.9 minutes in the sham, 0 Gy, 0.5 Gy, and 2 Gy groups. There was a statistically significant difference among groups (p = 0.006), as indicated by one-way ANOVA (Fig. 9).

**Histopathology**

In the histopathological assessment, the irradiated adrenal glands showed characteristic RT injury features in the adrenal cortex, including moderate hypertrophy, disorganization, cellular aggregates, increased vasculogenesis, condensed chromatin in the nucleus, and cytoplasmic swelling, which were most prominent in the zona fasciculata and medulla (Fig. 10).

**Discussion**

In this study, we intended to illustrate the correlation between AIR and fatigue. Fatigue after AIR was observed with decreased cortisol and increased ACTH. Further histopathology data showed characteristic RT injury features in the irradiated adrenal glands.

There is a relative lack of data regarding AIR-induced biological effects, which have only been mentioned in some case series. Casamassima et al. proposed the experience of stereotactic body radiation therapy (SBRT) for adrenal gland metastases at the University of Florence. Adrenal insufficiency after SBRT was documented as grade II in 1 of 48 patients [24]. Rubra et al. reviewed a series of 10 patients with adrenal metastases treated by SBRT at the University of Chicago. One patient developed grade II adrenal insufficiency 2.5 years after the completion of SBRT [25]. Wardak et al. reported one patient with bilateral adrenal gland metastases, which were all treated with SBRT. Blood analysis showed significantly increased ACTH levels and decreased cortisol levels, which raised concerns about primary adrenal insufficiency [26]. Chance et al. documented 43 patients with 49 adrenal metastases treated by SBRT. At a median dose of 60 Gy in 10 fractions, 50% of patients with bilaterally treated adrenal glands developed low-grade adrenal insufficiency after SBRT to adrenal metastases without acute high-grade toxicity [27].

Yuan et al. retrospectively analyzed 81 patients who received RT for adrenal metastases from HCC. The median radiation dose was 50 Gy, with a median fraction size of 2 Gy. Twenty-three patients reported grade I fatigue, and six patients reported grade II fatigue [28]. Mohsike et al. performed radio ablation of adrenal gland metastases with interstitial high-dose-rate brachytherapy in 37 patients. The median biological equivalent dose (BED) was 37.4 Gy. Grade I or II toxicities occurred in 11 patients (29%), including pain, nausea, vomiting, and fatigue. One grade 3 event (bleeding) occurred (3%). Ongoing cortisol substitution after treatment was required in two patients, while one patient required intermittent cortisol substitution for 1-month post-treatment [29]. König et al. evaluated 28 patients administered SBRT for adrenal gland metastases. The median BED was 75 Gy. The most common acute side effect was fatigue, two patients with grade I, and four patients with grade II. Late toxicity occurred in only three
patients. Two patients each suffered from grade I and II fatigue, and one patient had grade I gastrointestinal toxicity [30].

There is sparse literature considering the adrenal glands as organs at risk in RT. The evaluation of toxicity profiles was mostly from SBRT for adrenal metastases [31-33]. We regarded healthy adrenal glands, but not metastatic ones, as essential organs to spare in RT planning, which differed from previous studies. AIR might be related to adrenal insufficiency or fatigue in previous studies. However, no previous clinical data addressed the direct relationship of AIR, adrenal insufficiency, and fatigue, and monitoring of the HPA axis was not performed or incomplete in most studies. There was a full consideration of the HPA axis and circadian rhythm in the blood analysis in our clinical data, and FSS was used for patients to respond to their extent of fatigue. Furthermore, we developed an experimental animal model for mice AIR with histological proof of the effects of radiation and cortisol.

Other possible etiologies causing fatigue, such as electrolyte imbalance, infections, and mental state, remain to be excluded. In our study, all mice were fed sufficient food and purified water. No body weight loss was noted before and after the experiment. However, they lived in the cages and possibly with the stress and bad memory of blood harvest after their first experience. We observed the mice twice a day (day and night), and decreased activity and pain were noticed only a few days after surgery and RT. The mice recovered soon without specific unhealthy signs such as changes in bowel habits or unhealed wounds. The swimming endurance test was then applied with heat lamps and blankets prepared. Despite these precautions, we could not confirm the health and infection status of the mice. When it comes to humans, the etiologies of fatigue are much harder to evaluate due to different health statuses, socioeconomic status, family background, and personality.

There were several limitations to our study. First, the causal relationship between AIR and fatigue, which was assessed by decreased swimming endurance time, remained undetermined and may require further investigation. Secondly, the number of patients in our clinical observation with assessable cortisol and ACTH serum levels was too small to draw a firm conclusion. A third limitation is the timing and duration of AIR-induced biological effects may need a precise and long-term follow-up for evaluation in a large-scale study. Finally, other possible etiologies that cause fatigue should be examined in future work.

This investigation is the first study in which AIR-induced biological effects have been verified by an experimental animal model. Our data showed that low doses of ionizing radiation (single shot of 0.5 and 2 Gy) moderately impacted swimming endurance time and cortisol levels. These novel findings provide critical information for clinical dose-painting RT, such as IMRT, SBRT, and intensity-modulated proton therapy (IMPT), with possibly ignored adverse effects on both fatigue and the HPA axis. The development of a new constraint for RT planning for adrenal glands might be feasible.

Conclusions

Our preliminary clinical observations of fatigue development and the accompanying characteristic hormone alterations indicate that the adrenal glands could be regarded as an organ at risk from RT. This
observation is supported by experimental animal models with functional and histopathological evidence. However, considering the small number of cases reviewed, a larger prospective study is required for future work, and further clinical investigations to validate our findings are warranted.

**Abbreviations**

AIR: Adrenal ionizing radiation; ACTH: Adrenocorticotropic hormone; BED: Biological equivalent dose; CT: Computed tomography; CRH: Corticotropin-releasing hormone; DVHs: Dose-volume histograms; ELISA: Enzyme-linked immunosorbent assay; FSS: Fatigue severity scale; HCC: Hepatocellular carcinoma; HPA: Hypothalamic–pituitary–adrenal; IMPT: Intensity-modulated proton therapy; IMRT: Intensity-modulated radiation therapy; RT: Radiotherapy; SBRT: Stereotactic body radiation therapy

**Declarations**

**Ethics approval and consent to participate**

This study was approved by our ethical review committee with written informed consent provided by the patients.

**Consent for publication**

Figures depicting individual person's data were unidentifiable and there were no details on individuals reported within the manuscript. Therefore, separate consent for publication was not deemed necessary for the figures shown in this manuscript.

**Availability of data and materials**

The datasets during the current study were available from the corresponding author on reasonable request.

**Competing interests**

The authors declared that there were no conflicts of interest.

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**Authors' contributions**

Yu-Ming Huang contributed to the conceptualization, data curation, formal analyses, investigation, methodology, and writing of the original draft of this manuscript. Chih-Wen Chi contributed to the conceptualization, data curation, research, the writing of the literature review included in the manuscript,
and the editing of the first draft. Pao-Shu Wu, Hung-Chi Tai, and Ming-Nan Chien contributed to the methodology. Yu-Jen Chen helped with the conceptualization, funding acquisition, methodology, supervision of the experiments, literature review, and the writing and editing of the manuscript.

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References


Figures
Figure 1

RT plan for one patient with a mean dose of 44.5 Gy to the left adrenal gland. The y-shaped left adrenal gland was contoured with a dark green line in the planning CT images. Dose distributions and DVHs were recorded for evaluation. The dose distribution of the left adrenal gland was outlined by a dark green line in cumulative DVH. RT, radiotherapy; CT, computed tomography; DVH, dose-volume histogram.
Figure 2

The experimental animal model. (a) The left adrenal gland was surgically explored and exposed to RT. (b) After positioning was confirmed by the laser system, intraoperative RT was administered to the left adrenal glands of mice using a linear accelerator with an electron beam. A light field was used for adrenal gland localization. (c) All mice underwent a swimming test for endurance assessment as a representation of fatigue.
Figure 3

The serum levels of (a) morning cortisol, baseline cortisol, (b) aldosterone, and (c) ACTH. Baseline cortisol levels significantly declined after RT ($p = 0.002$). Alterations in the levels of morning cortisol and aldosterone showed a similar trend to baseline cortisol. The ACTH levels significantly increased after RT ($p = 0.007$), indicating possible compensatory feedback from the HPA axis. ACTH, adrenocorticotropic hormone; HPA, hypothalamic–pituitary–adrenal.
Figure 4

The effect of circadian rhythms on cortisol levels. The difference between morning and baseline cortisol levels was insignificant ($p = 0.62$) before RT. After RT, there was a significant difference between morning and baseline cortisol levels ($p = 0.02$). The circadian rhythm seemed to exert a greater effect after RT.

Figure 5

The effect of RT on FSS mean score. The FSS mean score increased after RT ($p = 0.02$).
The fatigue severity scale of all patients. The FSS score slightly increased after RT (p = 0.11), which indicated that fatigue developed more after RT. FSS, fatigue severity scale.

Fig. 6.

![Graph showing baseline cortisol levels before and after RT](image)

**Figure 6**

Serum levels of baseline cortisol. The levels of baseline cortisol declined after RT, especially in the RT 2 Gy group (p = 0.007) by the paired t-test.

Fig. 7.

![Graph showing evening cortisol levels before and after RT](image)
Figure 7
Serum levels of evening cortisol. The levels of evening cortisol significantly increased in the sham group (p = 0.04), whereas the levels in the 0.5 Gy and 2 Gy groups decreased with borderline significance (p = 0.05).

Fig. 8.

(a)

(b)
Circadian rhythms of cortisol levels. The circadian rhythm was not apparent (a) before and (b) after the experiment in all groups.

Fig. 9.

![Swimming endurance time](image)

**Figure 9**

Swimming endurance time. The swimming endurance time decreased in a dose-dependent manner by RT. There was a statistically significant difference among the groups ($p = 0.006$) by one-way ANOVA.

Fig. 10.
Figure 10

Histopathological changes induced by RT, as indicated by H&E staining. (a) Histopathology of the left adrenal gland from one of the 0 Gy groups. (b) Histopathology of the left adrenal gland from one of the 2 Gy groups. The irradiated adrenal glands showed characteristic RT injury features in the adrenal cortex, including moderate hypertrophy, disorganization, cellular aggregates, increased vasculogenesis, condensed chromatin in the nucleus, and cytoplasmic swelling, which were more prominent in the zona fasciculata and medulla. H&E, hematoxylin and eosin.