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Significance of Elevated HMGB1 Expression in Pituitary Apoplexy

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Abstract. *Background/Aim: High-mobility group box 1 (HMGB1) is a nuclear DNA-binding protein that exerts a range of proinflammatory actions when it is secreted extracellularly. We hypothesized that HMGB1 released from damaged cells in pituitary apoplexy would exacerbate the neurological symptoms due to acute inflammation. Patients and Methods: All the patients included in this study suffered from non-functioning pituitary adenoma. Four patients with apoplexy and three patients without apoplexy were included in this study. They underwent endonasal transsphenoidal endoscopic surgery to resect the tumors. We conducted enzyme-linked immunosorbent assay (ELISA) to measure HMGB1 in the surgical specimens. Results: Patients with apoplexy expressed HMGB1 at significantly higher levels than those in the non-apoplexy group (p=0.0478). Conclusion: HMGB1 may be involved in subacute inflammation of pituitary apoplexy. Further work is needed to elucidate the detailed biological significance of HMGB1 in this disease.*

High-mobility group box 1 (HMGB1) is a nuclear DNA-binding protein that plays essential roles in gene transcription and homeostasis. When cells are damaged, HMGB1 is secreted extracellularly and exerts a range of proinflammatory actions (1, 2). HMGB1 is also upregulated in tumor tissues and induces tumor cell proliferation (3, 4). Moreover, when HMGB1 is released from tumors, it suppresses immune responses and promotes tumor expansion, invasion, and/or metastasis (5, 6).

Pituitary glands sometimes suffer from adenoma which may cause hemorrhage or infarction, a condition defined as pituitary apoplexy. Patients with pituitary apoplexy exhibit sudden-onset of symptoms such as headache, visual disturbance, and endocrine dysfunction (7, 8). We hypothesized that HMGB1 released from damaged cells in pituitary apoplexy would exacerbate the neurological symptoms by causing acute inflammation. To address this hypothesis, we collected tumor specimens from pituitary apoplexies and evaluated the expression levels of HMGB1 as an early-phase immune stimulator.

Patients and Methods

Pituitary apoplexy and tissue collection. This study was approved by the ethical committee of Kindai University Faculty of Medicine. All the patients included in this study suffered from non-functioning pituitary adenoma. Pituitary apoplexy was defined as a case exhibiting a sudden onset of symptoms along with heterogeneous mass lesions within the pituitary gland detected on MRI (magnetic resonance imaging); those who only exhibited these imaging signs without having any symptoms were excluded. Four patients with apoplexy and three patients without apoplexy (controls) were included in this study. All the patients underwent endonasal transsphenoidal endoscopic surgery to resect tumors.

Enzyme-linked immunosorbent assay (ELISA). The procedure has been described previously (9). Briefly, surgical specimens were obtained from pituitary tumors in the operating room. The tissue samples were minced, and 0.5 mg of tissue per case were soaked in 500 μl PBS for 4 h. The sample supernatants were applied to HMGB1-specific ELISA (Shino-Test, Tokyo, Japan) as per the manufacturer's instructions. Initially, microplates were pre-coated with an anti-HMGB1 antibody. Diluted samples and standard samples were added to each well and incubated overnight at 37˚C. After washing 5 times, peroxidase-conjugated antibodies were added and incubated for 2 h at 25˚C. After additional 5 washes, tetramethylbenzidine solution was added and incubated for 30 min at room temperature. Finally, stop solution was added, and the optical density was measured photometrically at 450 nm using a multiple-plate reader. The assays were carried out in duplicate.

Statistics. Statistical analysis was performed as described previously (9, 10). Briefly, the Mann-Whitney *U*-test was performed to analyze differences in two groups. *p*-Value <0.05 was considered statistically significant.

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Key Words: Pituitary apoplexy, HMGB1, pituitary adenoma.

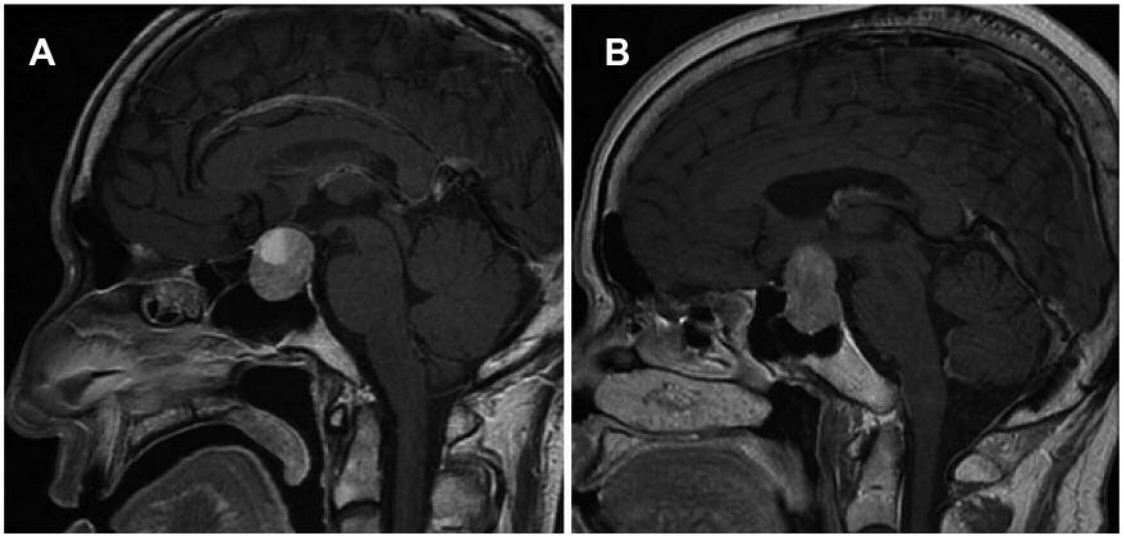

Figure 1. *MR images of pituitary apoplexy. MR images were obtained before surgery. A: Case 2: A patient with pituitary apoplexy. T1-weighted gadolinium-enhanced MRI (sagittal image) shows fresh blood associated with fluid-fluid level formation within the tumor. B: Case 6: A patient without pituitary apoplexy. T1-weighted gadolinium-enhanced MRI (sagittal image) shows the pituitary tumor without apoplexy.*

Table I. *Patient characteristics.*

No.	Apoplexy	Age	Gender	MRI findings	Tumor diameter (mm)
1	(+)	51	Male	Hemorrhage with niveau	34
2	(+)	42	Male	Hemorrhage with niveau	26
3	(+)	79	Female	Hemorrhage with niveau	16
4	(+)	38	Female	Hemorrhage with niveau	20
5	(–)	50	Female	No hemorrhage and/or infarction	24
6	(–)	66	Female	No hemorrhage and/or infarction	31
7	(–)	70	Male	No hemorrhage and/or infarction	17

Results

Patient details are provided in Table I. The mean diameter of the tumors was 24 mm (range=16-34 mm); the mean duration from pituitary apoplexy to surgery was 24 days (range=7-43 days). MRI demonstrated that pituitary apoplexy exhibited a liquid surface within the tumor masses, which indicates a fresh hematoma (Figure 1A). In contrast, pituitary tumor without apoplexy exhibited no hemorrhagic changes within the tumor as visualized by MRI (Figure 1B).

HE-stained histopathological sections of pituitary apoplexy demonstrated the infiltration of immune cells within the tumor masses (Figure 2A). In contrast, pituitary tumors without apoplexy exhibited no remarkable infiltration of immune cells (Figure 2B).

These findings led us to evaluate the expression levels of HMGB1 in the tumor lesions. To this end, we conducted HMGB1-specific ELISA (Figure 3). The apoplexy group exhibited significantly higher levels of HMGB1 compared with the non-apoplexy group (p=0.0478).

Discussion

We have previously shown that HMGB1 levels in the peripheral blood were elevated in stroke patients and can be used as a surrogate marker for their functional outcome (9). After that, we have shown that macrophage-mediated immune responses occurred in the lesions of cerebral hemorrhage (11) and cerebral infarction (12). In this study, we focused on pituitary tumor apoplexy as a unique research subject because this disease condition is relevant to both stroke and brain tumors (Figures 1 and 2). As we hypothesized, HMGB1 appeared to be involved in the subacute inflammation (Figures 2 and 3).

Symptoms of pituitary apoplexy can be primarily divided into visual dysfunction and endocrine dysfunction.

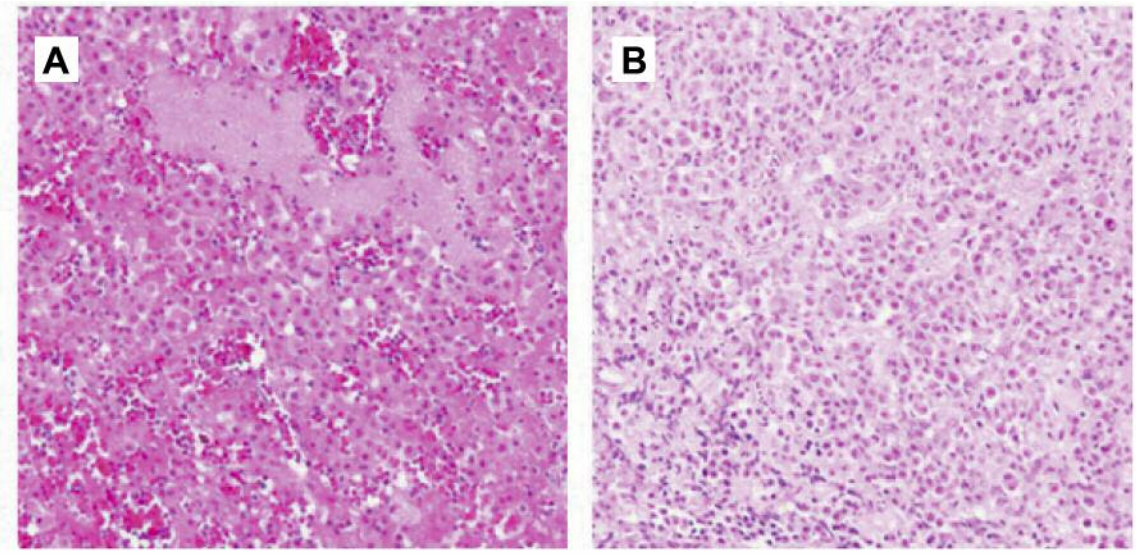

Figure 2. *Histopathological views of pituitary apoplexy. Histopathological analyses were conducted after surgery. A: Case 2: A patient with pituitary apoplexy. Pathological findings indicate proliferating cells with oval nuclei, and signs of hemorrhage and necrosis. B: Case 6: A patient without pituitary apoplexy. Pathological findings indicate proliferating cells with oval nuclei.*

Conventionally, symptomatic patients, particularly those exhibiting visual field disturbance or ocular movement disturbance, received emergency surgery. Increased pressure on the optic nerve is the primary cause of visual field disturbance, and the oculomotor nerves causes ocular movement disturbance. Surgical treatment aims to decrease peri-pituitary pressure by removing tumor masses and/or hematomas. As a result, the symptoms usually recover rapidly even during the chronic phase (13). In contrast, recent data have shown that surgical treatment is not beneficial to those with endocrine dysfunction as there was no difference in the functional outcome between patients with endocrine dysfunction who underwent surgery and those treated conservatively (13). Instead, approximately 80% of patients with pituitary apoplexy required hormone-replacement therapy regardless of surgical treatment. In addition, unlike patients with visual dysfunction, those with endocrine dysfunction appear to derive little benefit from surgical treatment. In this regard, our data showed that HMGB1 levels significantly increased in patients with pituitary apoplexy (Figure 3), which led us to hypothesize that HMGB1 may be the primary cause of endocrine dysfunction in pituitary apoplexy. That is, HMGB1 may be released from damaged cells in pituitary apoplexy to induce various inflammatory responses, which would result in hypophysitis. Further studies with a higher number of patients need to be conducted to elucidate this research question.

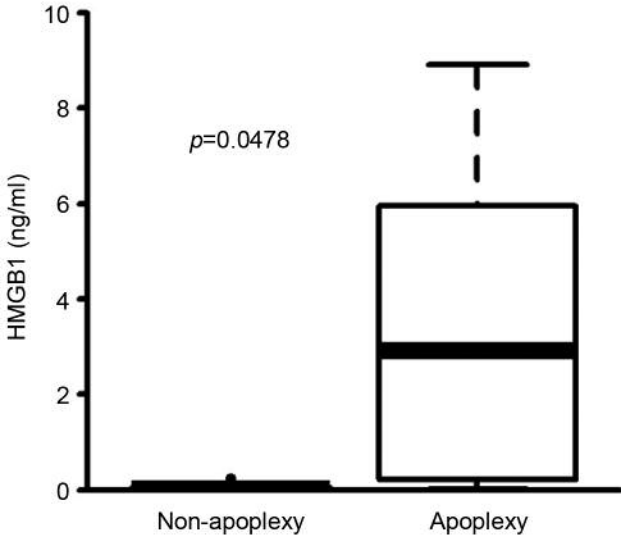

Figure 3. *HMGB1 levels in pituitary apoplexy. HMGB1 levels in pituitary lesions were measured by a specific ELISA. The apoplexy group exhibited significantly higher levels of HMGB1 compared with the non-apoplexy group (p=0.0478).*

Regarding the detailed immunological analyses, we have recently made use of ImmQuant (14). ImmQuant is a software that is freely available online (http://csgi.tau.ac.il/ImmQuant/). This software can visualize inferred alterations in the composition of immune cell populations in human samples based on microarray data to predict immune responses in given samples (15). This system seems suitable

for the current study, so we are planning to extract mRNA from pituitary tissues to conduct microarray and ImmQuant-based analyses.

In conclusion, HMGB1 appeared to be involved in subacute inflammation during pituitary apoplexy. We are in the process of elucidating the significance of HMGB1 in this disease condition.

Conflicts of Interest

The Authors declare that they have no conflicts of interest regarding this study.

Authors' Contributions

Conceptualization: TO and MF; Methodology: TO and MF; Resources: TO; Data curation: TO and MF; Writing-original draft preparation: TO; Writing-review and editing: MF; Supervision: AK; Project administration: TO, MF and AK.

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