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Relationship between MRI features and HIF-1α, GLUT1 and Ki-67 expression in pituitary adenoma with cystic degeneration

Fangfang Zhang[1†], Zhenhong Pan[2†], Jianwu Wu[3,4*] and Yinxing Huang[3,4*]

Abstract

Background Pituitary adenomas (PAs) are prevalent tumors that often exhibit ischemia, hypoxia, and cystic transformations, impacting their prognosis. The relationship between cystic degeneration in PAs and the expressions of hypoxia-inducible factor-1α (HIF-1α), glucose transporter 1 (GLUT1), and Ki-67 remains unclear. This study aims to analyze the correlation between MRI characteristics of cystic PA and the expression of these proteins.

Methods This is a retrospective analysis. A total of 74 patients with cystic PA and 30 PA patients without cystic degeneration were enrolled. Their MRI signs were analyzed. According to the T2WI signs of PA, they were divided into the fluid level group ($n=26$), non-fluid level group ($n=48$), and non-cyst group ($n=30$). Immunohistochemistry was performed to evaluate the expression levels of HIF-lα, GLUT1, and Ki-67 protein. Univariate and multinomial logistic regression analyses were used to evaluate the factors affecting MRI signs of PA. Spearman correlation was also performed.

Results There was no significant difference in gender, age, and HIF-1α protein expression among the three groups. Significant differences were found in invasiveness ($P=0.008$), GLUT1 ($P<0.001$), and Ki-67 protein expression ($P=0.009$) among the three groups. Pairwise comparisons revealed statistically significant differences in invasiveness, GLUT1, and Ki-67 protein expressions between the non-fluid level group and the non-cyst group. Furthermore, GLUT1 protein expression was significantly different between the non-fluid level group and the fluid level group. Notably, GLUT1 was identified as an independent factor for the non-fluid level cystic characteristics of PA. Additionally, GLUT1 was positively correlated with invasiveness and Ki-67.

Conclusion The non-fluid level cystic PA has higher invasiveness and higher proliferation than fluid level cystic PA and non-cyst PA, which may be related to high glucose metabolism as indicated by GLUT1 expression.

Keywords Pituitary adenoma, Fluid level, GLUT1, HIF-1α, Ki-67

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Background

Pituitary adenoma (PA), a common tumor of the sellar region, is characterized by ischemia and hypoxia due to high proliferation and hypermetabolism, which can lead to changes of hemorrhage, necrosis, or cystic degeneration [1]. PA is frequently characterized by ischemia and hypoxia, and cystic transformation or infarction due to stroke or necrosis may eventually occur. Goel et al. showed that the radical resection rate of cystic PA was low and the recurrence rate was high [2]. However, Fukui et al. found that the recurrence rate of PA with hemorrhage and cystic degeneration was lower than those without hemorrhage [3]. Although these findings are inconsistent, they still suggest that cystic degeneration in PA is closely related to its prognosis.

PA with cystic degeneration is manifested as fluid-level or non-fluid-level patterns in MRI. PA without fluid level is mostly manifested as a low signal in the T1WI image and a high signal in the T2WI image of MRI. However, the characteristics of cystic PA with fluid level are associated with time, and the signal of lower cystic fluid is higher than that of upper cystic fluid in the T2WI image, which is related to the deposition of hemosiderin after adenoma hemorrhage [4, 5].

Glucose transporter 1 (GLUT1) is the most abundant in the brain and plays a key role in glucose energy metabolism [6]. It has been shown that elevated glucose levels in cancer cells can be attributed to upregulated GLUT1 protein levels, while, decreased GLUT1 expression results in low cell proliferation, glucose uptake, tumor migration, and invasion [7, 8]. Hypoxia-inducible factor-1α (HIF-lα) is the only key protein that plays a regulatory role in tissue hypoxia, which can reflect the degree of tumor hypoxia and regulate GLUT1 and other proteins to inhibit aerobic glycolysis [9]. However, there is no report on HIF-lα and GLUT1 expression in PA with fluid-level and non-fluid-level cysts. Ki-67 is a protein expressed in the cell proliferation cycle and is currently the gold standard for evaluating the PA proliferation ability [10, 11]. However, there are currently no studies focusing on the correlation between cystic degeneration of PA and Ki-67 expression.

Herein, we evaluated the relationship between MRI signs of cystic PA and the expressions of HIF-lα, GLUT1, and Ki-67. This study may help understand the differences in hypoxia, metabolism, and proliferation of PA under different MRI signs of cystic degeneration.

Methods

Ethics

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the 900th Hospital of the Joint Logistics Team. Written informed consent from enrolled patients was waived due to the retrospective nature of the study and anonymized clinical data.

Patients

In this study, we enrolled PA patients who were treated in the Neurosurgery Department of our hospital from January 2019 to September 2020. Inclusion criteria: (1) patients had a postoperative pathological diagnosis of PA; (2) patients received preoperative MRI examination. Exclusion criteria: (1) patients with a history of metabolic disease, secondary surgery, radiation therapy, drug therapy, and other PA lesions; (2) Patients with other pituitary lesions such as Rathke cyst; (3) Patients with incomplete pathological and imaging data; (4) Patients complicated with serious blood system diseases and other systemic diseases; (5) Patients with long-term use of drugs that may affect coagulation function. Their clinical data were retrospectively analyzed.

MRI examination

The enhanced pituitary MRI scan via a Siemens 3.0T magnetic resonance machine (Siemens AG, Germany) was conducted for each patient. Scan sequences included coronal, axial, and sagittal scans onT1-weighted imaging (T1WI) and T2WI as well as three-dimensional contrast-enhanced scans. The MR scanning parameters included: fast spin echo sequence, TR 400–500 ms, TE 8–15 ms, and 3 excitations for T1WI; and, fast spin echo sequence, TR 3000 ms, TE 83–98 ms, and 2 excitations for T2WI; scanning field of view 180 mm × 180 mm; matrix 320–384 × 240–252, axial scanning slice thickness 5 mm and slice spacing 6.5 mm; scanning slice thickness 2.5 mm; and, slice spacing 2.75 mm. The contrast agent was gadopentetate meglumine, and the dose was 0.2 mmol/kg body weight.

MRI image analysis and patient grouping

The MRI data was collected from the INFINITT PACS system (Infineon, Seoul, Korea). The preoperative MRI data were evaluated by two neurosurgery physicians and two neuroradiologists. The MRI features of cystic degeneration in PA were evaluated. Knosp-Steiner classification was used [12]. PAs with Knosp grades 0–2 were classified as non-invasive and those with Knosp grades 3–4 were classified as invasive.

The PA patients were grouped according to the signs of PA on T2WI (Fig. 1). Patients with cysts > 2 mm and with fluid level on T2WI were defined as the fluid level group, whereas patients with cysts > 2 mm but without fluid level on T2WI were defined as the non-fluid level group. Patients without cysts on T2WI were defined as the non-cyst group.

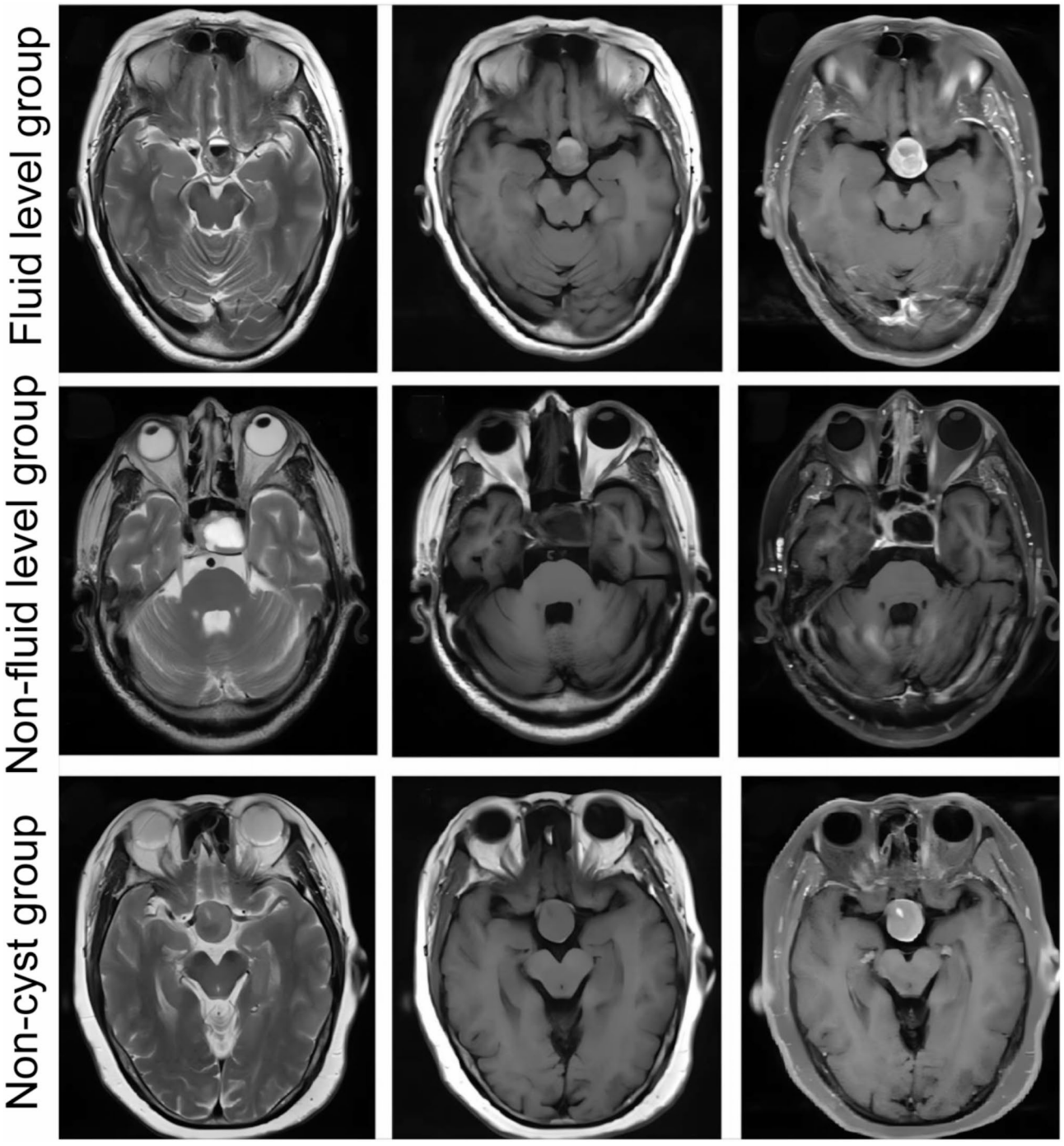

Fig. 1 Representative MRI images of PA. In the fluid level group, fluid level was observed in the cyst. The cyst showed high/low signal stratification on T2WI, high signal on T1WI, and isointensity on enhanced T1WI. The tumor parenchyma was enhanced on enhanced T1WI. In the non-fluid level group, the cyst showed a high signal on T2WI, but a low signal on T1WI and enhanced T1WI. The tumor parenchyma showed isointensity on T1WI and partial enhancement on enhanced T1WI. In the non-cyst group, no cystic degeneration was found on T2WI, T1WI, and TWI enhancement

Immunohistochemical staining

The intra-operatively collected PA specimens were sectioned into 4-μm slices. Immunohistochemical staining was performed as previously described [13]. Briefly, after deparaffinization, hydration, antigen retrieval, and blocking with goat serum, the sections were first incubated with anti-GLUT1 antibody (1:200, Abcam), anti-HIF-1α (1:100, Abcam), and anti-Ki-67 (1:200, Abcam) at 4℃ overnight and then incubated with corresponding secondary antibodies at 37℃ for 30 min. The sections were stained with DAB solution and hematoxylin sequentially. Representative images of immunohistochemistry staining

were acquired under the OLYMPULS BX-51 optical microscope (OLYMPULS, Tokyo, Japan) and evaluated by Image Pro Plus software 6.0 (Media Cybernetics, CA, USA). The staining intensity was scored as follows: 0 = absence of staining; 1 = weak staining; 2 = moderate staining; and 3 = strong staining. The percentage of positive staining cells was scored as follows: 0 = 0–10% of staining; 1 = 10–25% staining; 2 = 25–50% staining; 3 = ≥ 50% staining. The final score was obtained by multiplying the staining intensity score and percentage score. The final score > 2 was defined as a positive expression, and < 2 as a negative expression [14].

Statistical analysis

All data were analyzed with SPSS 23.0 (SPSS Inc., Chicago, IL, USA). *P* value < 0.05 was considered statistically significant. Categorical variables were represented as numbers and frequencies, and analyzed by χ2 test. Bonferroni correction was used for multiple comparisons, and *P* value < α/n (n is the number of comparisons) was considered to be statistically significant. The univariate analysis was conducted using the χ^2 test to identify the factors affecting MRI features of PA cystic degeneration. The significant factors ($P < 0.05$) were subjected to multinomial Logistic regression analysis to identify independent factors. The correlation between the two variables was analyzed by Spearman correlation.

Results

Basic characteristics of patients

In total, 104 patients with PA were enrolled in this study, including 26 cases in the fluid level group, 48 cases in the non-fluid level group, and 30 cases in the non-cyst group. Among the 74 cases in the fluid level group and non-fluid level group, there were 38 males and 36 females. Their age ranged from 17 to 83 years old, with an average age of (54.42 ± 13.15) years old.

Expression of HIF-1α, GLUT1 and Ki-67 in PA tissues

The expression of HIF-1α, GLUT1, and Ki-67 in PA tissues was detected with immunohistochemistry (Fig. 2). HIF-1α positive expression presented as brown-yellow fine particle staining in the nucleus. The overall positive HIF-1α expression rate in 104 cases of PA was 43.27% (45/104). GLUT1 was positively expressed in the cytoplasm, with an overall positive expression rate of 43.27% (45/104). Additionally, Ki-67 was expressed in the nucleus, showing as brown-yellow fine particles, and the overall positive expression rate was 35.08% (43/104).

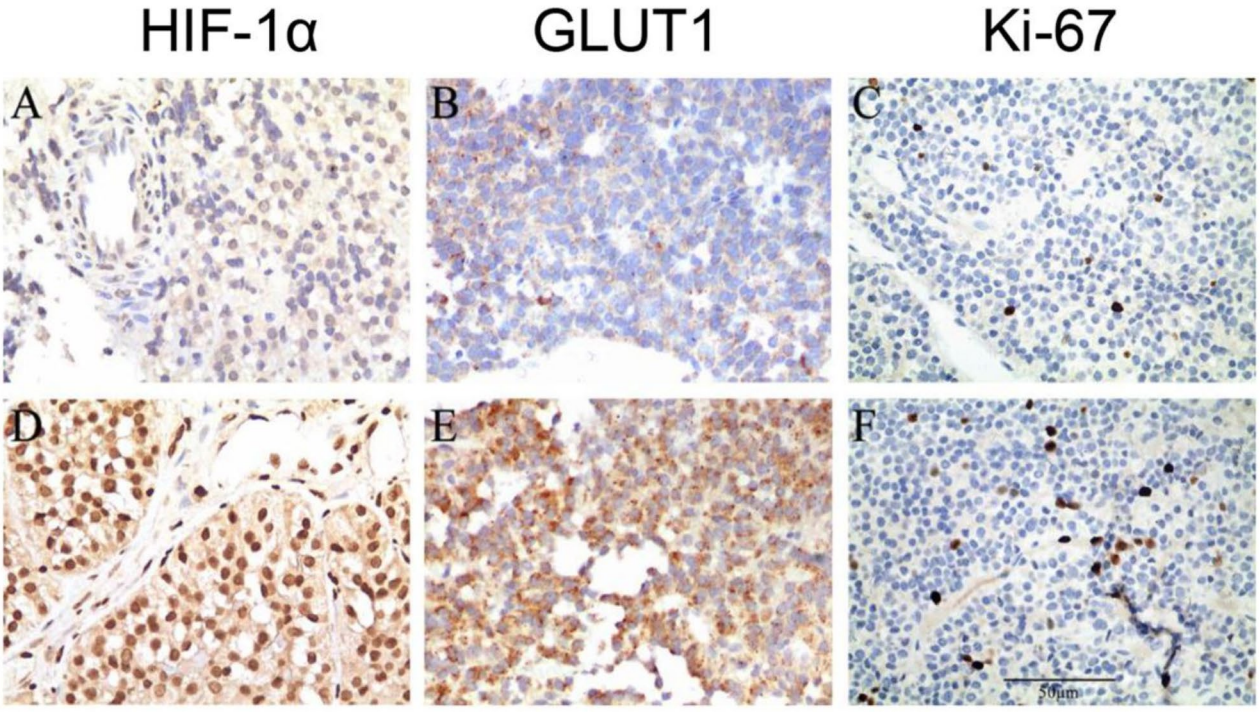

Fig. 2 Immunohistochemical staining of HIF-1α, GLUT1, and Ki-67 in PA paraffin specimens. **A**: HIF-1α negative expression (×200 times); **B**: GLUT1 negative expression (×200 times); **C**: Ki-67 negative expression (×200 times); **D**: HIF-1α positive expression (×200 times); **E**: GLUT1 positive expression (×200 times); **F**: Ki-67 positive expression (×200 times)

Table 1 Univariate analysis of the factors affecting MRI features of PA cystic degeneration

Characteristics	PA cystic degeneration		Non-cyst	χ^2	*P*
	Non-fluid level	Fluid level			
	n = 48	*n* = 26	*n* = 30		
Sex				0.832	0.660
Male	23 (47.92%)	15 (57.69%)	14 (46.67%)		
Female	25 (52.08%)	11 (42.31%)	16 (53.33%)		
Age, years				1.063	0.588
< 60	27 (56.25%)	17 (65.38%)	20 (66.67%)		
≥ 60	21 (43.75%)	9 (34.62%)	10 (33.33%)		
Invasiveness				9.650	0.008
Non	25 (52.08%)	20 (76.92%)	25 (83.33%)		
Yes	23 (47.92%)	6 (23.08%)	5 (16.67%)		
HIF-1α expression				1.899	0.387
Negative	30 (62.50%)	15 (57.69%)	14 (46.67%)		
Positive	18 (37.59%)	11 (42.31%)	16 (53.33%)		
GLUT1 expression				16.571	< 0.001
Negative	17 (35.42%)	19 (73.08%)	23 (76.67%)		
Positive	31 (64.58%)	7 (26.92%)	7 (23.33%)		
Ki-67 expression				9.476	0.009
Negative	21 (43.75%)	18 (69.23%)	22 (73.33%)		
Positive	27 (56.25%)	8 (30.77%)	8 (26.67%)		

Table 2 Multinomial logistic regression analysis of the independent factors affecting MRI features of PA with non-fluid level cyst and fluid-level cyst

Variable	β	SE	*P*	Exp(B)	95%CI
Non-fluid level					
GLUT1	-1.214	0.576	0.035	0.297	(0.096 ~ 0.919)
Ki-67	-0.954	0.553	0.085	0.385	(0.130 ~ 1.139)
Invasiveness	-1.130	0.621	0.069	0.323	(0.096 ~ 1.091)
Fluid level					
GLUT1	-0.044	0.665	0.947	0.957	(0.260 ~ 3.520)
Ki-67	-0.195	0.612	0.751	0.823	(0.248 ~ 2.732)
Invasiveness	-0.395	0.705	0.576	0.674	(0.169 ~ 2.686)

Note: SE, standard error; 95%CI, 95% confidence interval

Univariate analysis of the factors affecting MRI characteristics of PA cystic degeneration

To identify the factors affecting MRI features of PA cystic degeneration the univariate analysis was performed using the χ^2 test. As shown in Table 1, there was no significant difference in gender, age, and HIF-1α protein expression levels among the three groups (all $P > 0.05$). However, significant differences were observed in invasiveness, GLUT1, and Ki-67 protein expression (Bonferroni correction, P for invasiveness = 0.008, P for GLUT1 < 0.001, P for Ki-67 = 0.009, $\alpha/3 = 0.0167$) among three groups. Pairwise comparisons revealed statistically significant differences in invasiveness, GLUT1, and Ki-67 protein expressions between the non-fluid level group and the non-cyst group. Furthermore, a statistically significant difference in GLUT1 protein expression was noted between the non-fluid level group and the fluid level group. In contrast, no statistically significant differences were found among the other group pairs.

Multinomial logistic regression analysis of the independent factors affecting MRI characteristics of PA cystic degeneration

The factors with significant differences in the univariate analysis, including invasiveness, GLUT1, and Ki-67, were further subjected to multinomial logistic regression analysis. The results showed that GLUT1 was identified as an independent factor for non-fluid-level cystic changes in PA (Table 2).

Correlation between invasiveness, GLUT1, and Ki-67 in PA cysts

Spearman rank correlation analysis showed that GLUT1 expression levels were positively correlated with invasiveness and Ki-67 expression levels ($P < 0.05$) (Fig. 3).

Discussion

Currently, it is believed that PA cysts are caused by hemorrhage or ischemic necrosis [2, 4], and the mechanisms of different cystic signs are different. Cysts without fluid levels are mostly indicative of ischemic changes, and a few are caused by chronic bleeding, while cysts with fluid levels are mostly caused by intratumoral hemorrhage [15, 16]. These findings suggest that the MRI signs of cystic degeneration may be associated with different apoplexy types of PA. Fukui et al. [3] showed cranial neuropathy improvement was more common in PA apoplexy patients with conservative treatment, and there was spontaneous

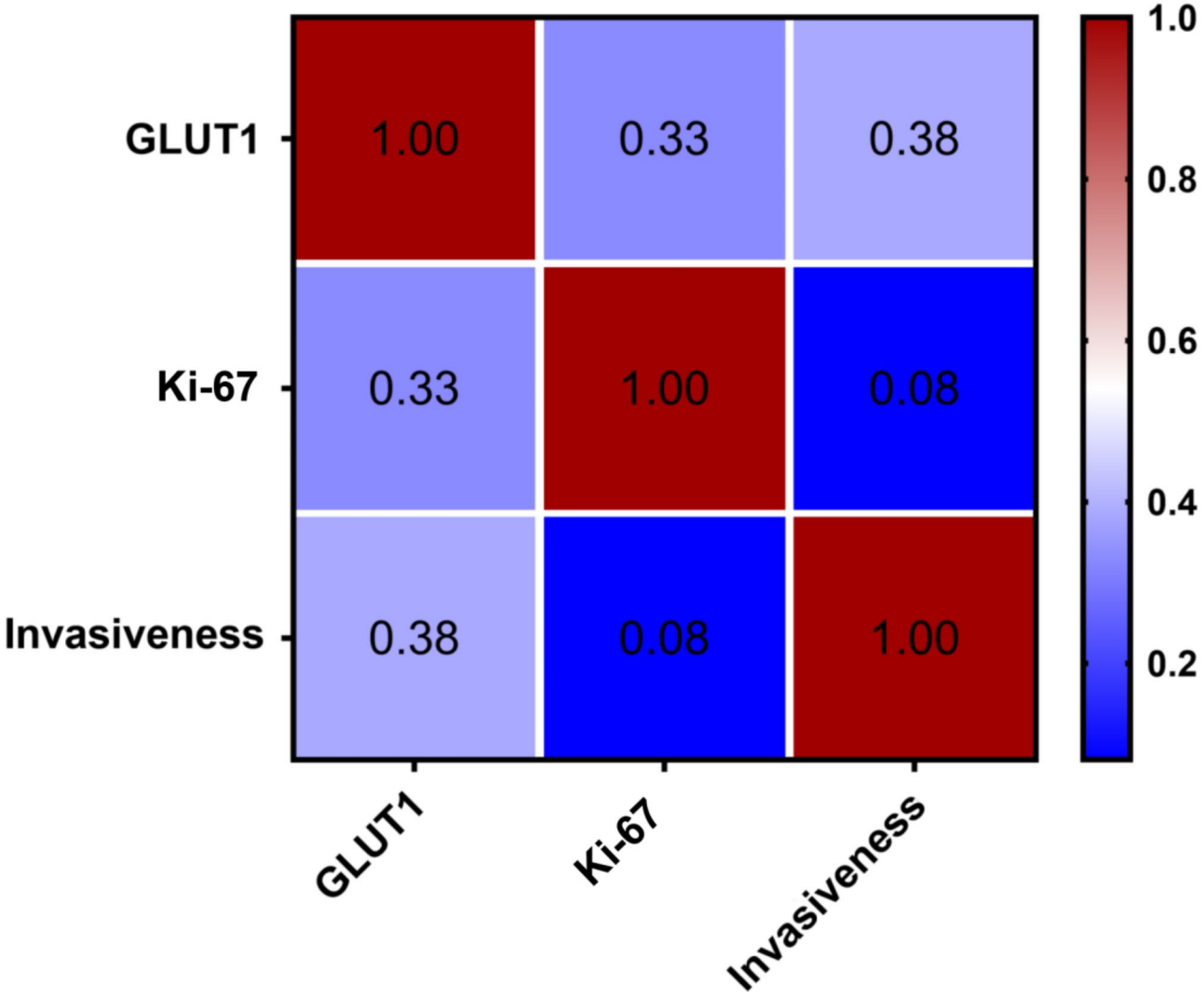

Fig. 3 Correlation analysis of GLUT1, Ki-67, and invasiveness in patients with PA cystic degeneration. Spearman correlation was conducted and the correlation coefficient was shown

regression of PA volume in patients with conservative treatment. Marx et al. also showed the effectiveness of conservative treatment for PA apoplexy patients [17]. However, Goel et al. showed that the total resection rate of PA with fluid level (a type of hemorrhagic cystic change) after apoplexy was lower and the recurrence rate was high [2]. These studies obtained conflicting results. The possible reason may be because Fukui et al. analyzed cystic changes according to bleeding and non-bleeding subtypes, while Goel et al. only analyzed the recurrence of PA in the fluid level group, and did not set up a control group. However, they both showed that the cystic changes of PA were related to PA recurrence and proliferation. In this study, we divided the participants into the non-fluid level group, fluid level group, and non-cyst group, and the results showed that the non-fluid level group had higher invasiveness and Ki-67 expression levels than the fluid level group and non-cyst group.

According to the Warburg effect, tumors still tend to provide energy and produce lactate through the glycolytic pathway even under aerobic conditions [18], which is closely related to the reduction of oxygen consumption and the improvement of hypoxia tolerance. Meziou et al. performed immunohistochemistry and 18 F-FDG-PET/CT in 94 prostate cancer patients and showed that GLUT1 expression in prostate cancer was associated with 18 F-FDG-PET/CT uptake and poor prognostic factors [19]. Meanwhile, a higher 18 F-FDG-PET/CT uptake rate represented a higher level of glucose metabolism in the tumor [19]. In this study, there was no significant difference in HIF-1α protein expression levels among PA groups (non-fluid level group, fluid level group, and non-cyst group), which may be related to the general hypoxia

in PA. The level of invasiveness, GLUT1 and Ki-67 protein expression in the fluid level group was higher than that in the fluid level group and the non-cyst group. GLUT1 was identified as an independent factor of non-fluid-level cystic features in PA. Ki-67 expression and invasiveness were positively correlated with GLUT1. These results suggest a higher level of glucose metabolism in the non-fluid level group, and this may account for its highly proliferative and invasive properties.

There were several limitations in this study. Firstly, the sample size of this study was too small to analyze the correlation between the pathological type and the grouping of PA cysts. Secondly, protein expression was evaluated only with immunohistochemical staining, but not other quantitative methods.

Conclusion

In this study, we found significant differences in the expression of GLUT1 between different signs of PA (non-fluid level group, fluid level group, and non-cyst group). The non-fluid level cystic PA had higher invasiveness and higher proliferation, which may be related to glucose metabolism, and should be removed as soon as possible.

Abbreviations

PA	Pituitary adenoma
HIF-Iα	Hypoxia-inducible factor-1α
GLUT1	Glucose transporter

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Not applicable.

Author contributions

Fangfang Zhang: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Supervision; Validation; Visualization; Writing - original draft. Zhenhong Pan: Data curation; Formal analysis; Investigation; Methodology; Software; Writing - original draft. Jianwu Wu: Data curation; Formal analysis; Investigation; Writing - review & editing. Yinxing Huang: Conceptualization; Funding acquisition; Project administration; Supervision; Writing - review & editing. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the 900th Hospital of the Joint Logistics Team. Written informed consent from enrolled patients was waived due to the retrospective nature of the study and anonymized clinical data.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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