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Serum AGR2 as a useful biomarker for pituitary adenomas

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ABSTRACT

Objective: This study aims to evaluate whether the serum Anterior Gradient-2 (AGR2) can be used as a potential biomarker screening in the diagnosis of Pituitary adenomas(PAs).
Patients and methods: The serum AGR2 protein levels were preoperatively measured in 163 PA patients, 43 patients with other sellar lesions excluding PAs, 7 patients with prostate cancer as a positive control and 20 normal people(10 female and 10 male) using Enzyme-Linked ImmunoSorbent Assay (ELISA). Differences in the serum AGR2 level between different groups were analyzed for statistical significance with a Mann-Whitney *U* test.
Results: The data showed that serum AGR2 level was significantly higher in the serum of PA patients (250.10 ± 79.14 ng/ml) than the patients with other sellar lesions (220.84 ± 79.62 ng/ml, P = 0.017) and normal people (163.67 ± 50.38 ng/ml, P < 0.001). Receiver operating characteristic (ROC) curve analysis was used. The detected area under the curve (AUC) was 0.835. The calculated optimal cut-off point for AGR2 level in serum samples was 158.63 ng/ml (Youden index = 0.564). The sensitivity was 91.4% and the specificity was 65.0%. Despite the variety of PA clinical features, the serum level of AGR2 are definite in PAs, although there may be a difference between male or female patients.
Conclusion: Our data suggests AGR2 as a potential biomarker for the diagnosis of PAs.

1. Introduction

Pituitary adenomas (PAs) account for about 15% of intracranial tumors, although autopsy series have found that the incidence of PAs in the general population may be as high as 25% [1]. Many PAs are incidentally found on imaging scans performed for unrelated reasons. PAs are not difficult to diagnose in other suffering from significant hormone hypersecretion, hypothalamic/pituitary dysfunction and visual field compromise due to their large size [2,3]. However, the opposite is true for the patients with PAs that are completely asymptomatic, and are incidentally found on magnetic resonance imaging (MRI) [4] .

MRI cannot make a definite diagnosis of PAs or nor differentiate them from other sellar lesions. Clinical non-functioning PAs without hormone abnormality and visual field compromise only need close follow-up. PAs that are large enough to cause symptoms can easily be completely eliminated before they invade the cavernous sinus [5,6]. The other sellar lesions, including craniopharyngioma, Rathke's cyst, or chordoma, have their own natural history and therapeutic strategies. Therefore, to investigating a screening biomarker is imperative for the diagnosis of PAs and differential diagnosis of PAs from other sellar lesions.

Anterior Gradient-2 (AGR2) is reported to be overexpressed in several adenocarcinomas [7–10]. It is considered to promote cell proliferation, cell survival, and metastasis of cancer cells. Salmans et al. demonstrated that AGR2 is a marker of breast cancer metastasis and overexpression in endoplasmic reticulum(ER)-positive breast cancer is associated with poor prognosis, particularly in tumors that escape anti-hormone therapies [11]. This indicates that AGR2 may participate in the process of cell metastasis in hormone-associated tumors. Pituitary adenomas are associated with multiple hormones in human beings. Serum AGR2 has been reported as an early diagnostic and postoperative prognostic biomarker of human lung adenocarcinoma [12].

In this study, we are going to study the serum AGR2 levels in PA patients and investigate whether AGR2 could be a potential diagnostic serum biomarker for human PAs.

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2. Materials and methods

2.1. Patients and serum samples

163 PAs, including 110 non-functioning(NF) adenomas, 29 (growth hormone)GH-secreting adenomas, 17 prolactin (PRL)-secreting adenomas, and 7 adenocorticotropic hormone (ACTH)-secreting adenomas were randomly selected from the patients operated between 2012 and 2014 in the Department of Neurosurgery, Jinling Hospital, School of Medicine, Nanjing University. 10 normal males and 10 normal females were selected as the control group. 43 patients with other sellar lesions excluding PA(including 13 of craniopharyngioma, 24 of Rathke's cyst, 3 of chordoma and 3 of phlogistic granuloma) were selected to compare with the PA patients. 7 patients with prostate cancer were selected as positive control.

Blood (10 ml) was drawn from each subject from a peripheral vein into EDTA tubes and centrifuged at 2000 rpm for 5 min. Serum samples were obtained and stored at −80 °C until analyzed.

2.2. Detection of AGR2 serum levels by ELISA

AGR2 levels in the serum samples were measured by ELISA using a AGR2 test kit (Uscn Life Science Inc., Wuhan, China) following the manufacturer's instruction. Briefly, AGR2 antibody was pre-added in plates followed by adding 100 μl of standard (100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0 ng/ml) and samples were added to the plates and incubated for 2 h at 37 °C. Then samples were then incubated with anti-AGR2 polyclonal antibody (biotin-conjugated) at 37 °C for 1 h. After incubation with avidin conjugated to horseradish peroxidase, a TMB substrate solution was added followed by incubation for 25 min in a dark room. Absorbance was measured at 450 nm using microplate reader (Bio-Rad, Shanghai, China). AGR2 concentrations were determined by comparing their optical density (OD) with the standard curve.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Chicago, IL, USA). Differences in the serum AGR2 level between different groups were analyzed for statistical significance with a Mann-Whitney *U* test. The receiver operating characteristic (ROC) curve analysis was used to detect the optimal cut-off point and determine the sensitivity and specificity of AGR2 for separating PA patients from non-PA controls. The area under the ROC curve (AUC) was calculated. P values <0.05 were considered to be statistically significant.

3. Results

3.1. Serum levels of AGR2 in pituitary adenoma patients

Using ELISA, AGR2 was detected in the blood serum samples as described in methods. As in Fig. 1 despicts, the AGR2 level was significantly higher in the serum of PA patients (250.10 ± 79.14 ng/ml) than that in normal control (163.67 ± 50.38 ng/ml, P < 0.001) and patients with other non-PAsellar lesions (220.84 ± 79.62 ng/ml, P = 0.017). Also, the AGR2 level was significantly higher in the serum of non-PA patients with other sellar lesions (220.84 ± 79.62 ng/ml) than that in normal control (163.67 ± 50.38 ng/ml, P = 0.002). This indicated that the detection of serum levels of AGR2 could distinguish not only PA patients from non-PA patients with other sellar lesions, but PA or non-PA patients from normal people as well.

We subsequently analysed the serum AGR2 level in different PA subtypes. As the results shown in Table 1, the mean level of AGR2 in GH secreting PAs (291.38 ± 97.37 ng/ml) is the highest, AGR2 level

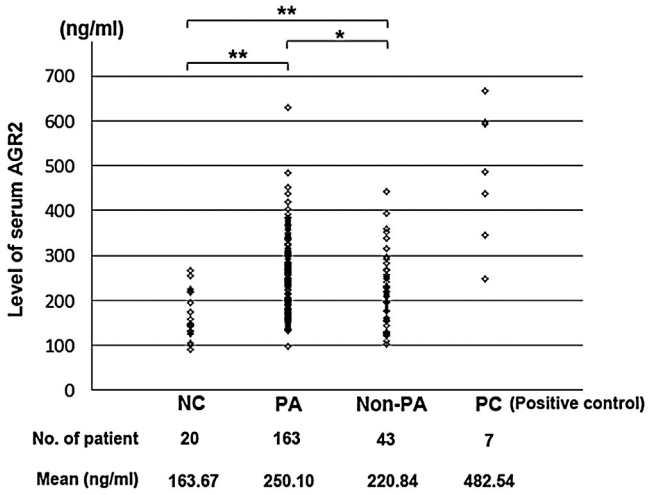

Fig. 1. Scatter plot of serum AGR2 levels in pituitary adenoma patients, normal controls, non-PA patients with other occupying lesions in saddle area and prostate cancer patients (positive control) detected by ELISA. **P<0.01, *P<0.05. NC: normal control; PA: pituitary adenoma; Non-PA: non-PA patients with other occupying lesions in saddle area; PC: prostate cancer.

Table 1
Serum levels of AGR2 in different subtypes of PAs. (AGR2, anterior gradient 2; PA, pituitary adenoma; PRL, prolactin-secreting; GH, growth hormone-secreting; ACTH, adenocorticotropic hormone-secreting; NF, non-functioning).

PA subtypes	No. of patients	AGR2 (ng/ml)	
		Mean	SD
PRL	17	215.81	44.12
GH	29	291.38	97.37
ACTH	7	232.58	76.03
NF	110	245.63	74.79
Total	163	250.10	79.14

in non-functioning PAs (245.63 ± 74.79 ng/ml) is higher than ACTH secreting PAs (232.58 ± 76.03 ng/ml) and the average level of AGR2 in PRL secreting PAs (215.81 ± 44.12 ng/ml) is the lowest. However, there is no significant difference of AGR2 level in different PA subtypes (P > 0.05).

3.2. The ROC curve analysis

The ROC curve was presented in Fig. 2. The detected area under the curve (AUC) was 0.835. The calculated optimal cut-off point for AGR2 level in serum samples was 158.63 ng/ml (Youden index = 0.564). The sensitivity was 91.4% (149 of 163 PA patients). whereas the specificity was 65.0% (13 of 20 normal controls). This indicated that the detection of serum AGR2 levels by ELISA in this study is appropriated with high diagnostic accuracy.

3.3. Association of the serum level of AGR2 with clinical features of PAs

Within the 163 PA patients, 83 of them were male and 80 were female; 75 of them were defined as cavernous sinus-invasive PAs, and others were non-invasive (according to Knosp's classification); 14 of them were recurrent PAs, and the others were primary; only 5 were microadenomas (diameter ≤ 10 mm), with the remaining classified as macroadenomas (diameter > 10 mm). The associations between clinical variables and serum level of AGR2 were shown in Table 2. The results showed a significant difference of AGR2 level in male and female patients (P = 0.047 < 0.05). The serum level of AGR2 was higher in male PA patients than in female. Interestingly, no difference was found in serum level of AGR2 between normal male