BENHA MEDICAL JOURNAL bmj

Print ISSN 1110-208X

Online ISSN 2357-0016

Original article

Diagnostic Significance of Expression of IGF2, SMAD4, PTTG1 and Clinicopathological Parameters in Differentiating Adrenocortical Tumors

Omneya Y. Bassyoni, Ebtehal M. Abdel-Aal

Department of Pathology, Faculty of Medicine Benha University, Egypt.

Corresponding to: Omneya Y. Bassyoni, Department of Pathology, Faculty of Medicine Benha University, Egypt.

Email:

omneyayoussef2015@gmail.com

Received:15 April 2024

Accepted:4 June 2024

Abstract

Among the most aggressive tumors is adrenocortical carcinoma. It is still a great challenge to distinguish between adrenocortical adenoma and carcinoma. For many tumors, IGF2 is essential to their growth and development. One of the genes that suppresses tumor growth is SMAD4 and one that may encourage tumorigenesis is PTTG1. **Aim of this work:** Evaluation of the clinicopathological factors, IGF2, SMAD4, and PTTG1 immuno-expression to distinguish adrenocortical tumors and, consequently, the therapeutic modalities. **Material and Methods**: A retrospective study was conducted on selected 40 cases of adrenocortical tumors on which immunohistochemical expression of PTTG1, SMAD4, and IGF2- were performed **.** Receiver operating characteristic (ROC) curves were performed to assess the diagnostic performance of the studied markers as well as clinicopathological variables in discriminating ACA from ACC. **Results:** Significant differences in tumor size, weight and high Weiss score- were important in differentiating ACA from ACC ($p < 0.001$) with 100% sensitivity & 60% specificity. The most sensitive and specific criterion was "mitotic rate >5/50 HPF" (100%). Only 5% of ACA cases had positive IGF2 ($p<0.01$) expression, compared to 80% and 75% of ACC cases that expressed IGF2 & PTTG1, respectively ($p < 0.01$). While, SMAD4 was negative in (80%) of ACC patients ($p < 0.05$). For identifying adrenocortical cancer, IGF2 had the best specificity and sensitivity (95% & 80%, respectively). In the current study, IGF2 significantly related positively with PTTG1 ($r=.898$). **Conclusion**: In adrenal cortical neoplasms, IGF-2, SMAD4, and PTTG1 may be used as promising diagnostic biomarkers with potential therapeutic applications. Differentiating between adrenocortical adenoma and carcinoma may be significantly aided by the combined expression of this panel of markers (IGF2, SMAD4, & PTTG1) and varying clinicopathological characteristics.

Keywords: Adrenocortical adenoma, Adrenocortical carcinoma, IGF2, SMAD4, PTTG1.

Introduction

Adrenocortical tumors (ACTs) are tumors of the adrenal cortex that affect 3–10% of adults[1]. Most of ACT are benign adrenocortical adenomas (ACA) that do not function; they were found by chance during imaging examinations done for unrelated therapeutic purposes [2].

Adrenocortical carcinoma (ACC), on the other hand, is an extremely aggressive and uncommon cancer that affects 0.7–2.0 cases per million people annually [3]. Benign tumors represented 9.01% of all suprarenal lesions in Egypt, whereas ACA made up 40.48% among the benign ones [4].

The histological diagnosis of adrenal tumor is still difficult. The diagnostic workup of adrenal lesions has been studied in many researches in recent years. Since the majority of adenomas are less than 5 cm in size, and the median size of ACCs is greater than 11 cm, size is an apparent criterion to distinguish an adrenal mass, but 3-10 cm adrenal tumors remain difficult to diagnose [5].

In order to distinguish benign from malignant adrenocortical tumors, we need to assess several criteria. The most widely used technique for determining malignancy is the Weiss system, which includes nine-point histopathological criteria [6].

It is suggested that the Weiss method requires a total score of three or more as a threshold for malignancy [7]. But, some tumors may behave in a malignant manner even though they were initially given a Weiss score of 2, and other tumors with a Weiss score of 3 may have a clinical benign course [8]. So, in addition to standard pathological analysis, a thorough understanding of the clinical and morphological features of adrenocortical adenomas and carcinomas is required, as well as the identification of trustworthy biomarkers [9].

Insulin growth factor 2 (IGF-2) overexpression and constitutive activation of the Wnt/β-catenin pathway- are the two most common changes found in ACC [10]. Growth factors like IGF-2, which are mostly released by the liver, are essential for the expansion and maturation of several tissues. It controls growth in general and in particular, fetal growth. The IGF1 receptor (IGF1R), insulin receptor (IR), and IGF2 receptor (IGF2R) modulate its effects [11]. The ability of many cancer cells to proliferate, survive, and spread is influenced by IGF2 [12].

One of the signal transduction proteins in the family of tumor suppressor genes is SMAD family member 4 (SMAD4), also known as deleted in pancreatic cancer 4 (DPC4). The inactivation of SMAD4 is most commonly associated with pancreatic cancer [13].

The pathway of TGF-β/SMAD4 signaling regulates the transmission of signals from the cell membrane to the nucleus and it is in charge of several

cellular functions, such as migration, apoptosis, differentiation, proliferation, and the initiation and spread of cancer [14].

The pituitary-tumor transforming gene (PTTG1), also known as securin, is regarded as an oncogene. It is known to influence angiogenesis, metastasis, and the response to treatment in cancer [15]. It can also induce carcinogenesis in a variety of malignancies. Many different types of malignancies like bladder and prostate cancer show high expression levels of PTTG1 [16, 17].

With the hope of developing novel diagnostic and therapeutic approaches for adrenocortical adenoma and carcinoma, the objective of this work is to assess the expression of IGF2, SMAD4, PTTG1, and other clinicopathological factors in adrenocortical tumors in order to clarify their actions.

Material and methods:

Study Groups:

This retrospective study was conducted on formalin-fixed paraffin embedded biopsy tissues from selected 40 cases of adrenal cortical tumors; 20 of which were classified as adrenocortical carcinoma (ACC) and 20 as adrenocortical adenoma (ACA). From January 2018 to December 2020, cases were collected from the Pathology Department's and the Early Cancer Detection Unit's (ECDU) archives at Faculty of Medicine, Benha University. The Ethical Committee of Faculty of Medicine, Benha University, approved this study (RC-17-1-2024).

The control cases were 10 cases of normal adrenal gland resected as part of radical nephrectomy for an adjacent renal cancer.

The availability of clinicopathological information taken from the patient files, such as; age, sex, laterality, tumor size, grade, lymph node status (N), distant metastasis (M), and stage- were taken as inclusion criteria. While, cases lacking clinicopathological information or paraffin blocks were not included in the current study.

A-Histopathological Examination:

Formalin fixed /Paraffin embedded blocks were sliced at 5 μm thickness and stained by hematoxylin and eosin stain. Without knowing the diagnosis, two observers reviewed the microscopic sections from all the cases. Two observers who were blind to the patients' clinical courses applied the Weiss approach to each of the 40 adrenal cortical tumors independently. Classification of adenomas and carcinomas was done using the Weiss criteria [6]. For the studied cases, the grading system was applied with a cut-off of 20 mitoses per 10 mm2 [18]. In accordance with AJCC, 8th edition [19], the ENSAT staging system- was used for the ACC patients.

B-Immunohistochemical Procedure:

The streptavidin-biotin technique was employed for immunohistochemical analysis in accordance with the guidelines provided by the manufacturer ***(Neomarker, LABVISION, USA, CA 94538-7310).*** Antigen retrieval by Citrate buffer at PH 6.0 was used .As a chromogen, the

slices were stained with 0.02% diaminobenzidine (DAB) solution. Sections were then dehydrated, mounted, and counterstained with hematoxylin. As indicated in **Table (1),** positive controls were added and negative controls were done by omitting the primary antibody.

Immunohistochemical assessment of IGF2, SMAD4 and PTTG1 expression within the studied cases:

In accordance with previous study [20], IGF2 immunostaining was categorized into four tiers based on cytoplasmic staining. As regard SMAD4, the cytoplasmic staining was evaluated as described by previous study [21]. Following prior investigation [22], we used a scoring method for PTTG1 that was based on the degree and intensity of nuclear staining.

Statistical analysis:

The statistical software SPSS **(SPSS Inc., Chicago, IL, USA) (version 22)** for Microsoft Windows was used to analyze the data as follows: Whereas P value >0.05 indicates non-significant (NS), P<0.05 indicates significance (S), P≤ 0.01 indicates highly significant (HS). To evaluate the efficacy of IGF2, SMAD-4, PTTG1, and variable clinicopathological characteristics in distinguishing patients with adrenocortical adenoma from carcinoma, receiver operating characteristic (ROC) curves were performed.

Results:

Clinicopathological features of the studied cases:

Forty cases of adrenal cortical tumors were used in this study. The age range of the ACA cases- under study- was 30 to 67 years, with a mean age of 49.2 ± 12.0 years, whereas the ACC cases- under study- had an age range of 46 to 85 years, with a mean age of 58.2 ± 10.8 years. The average case size for ACA was 2.9 ± 0.9 cm (with a range of 2–5 cm), while the average case size for ACC was 11.8 ± 3.9 cm (with a range of 5–16 cm).

Comparison of clinicopathological data among studied cases of ACA & ACC:

The size and weight of malignant adrenocortical tumors were notably greater than those of benign tumors (P < 0.001). Compared to benign tumors, malignant tumors had a considerably higher mean Weiss score (P < 0.001). When differentiating between adenoma and carcinoma, there was a statistically significant positive correlation with each Weiss score parameter (p <0.001), including nuclear grade, mitotic rate >5/50 HPF, atypical mitosis, necrosis, clear cells ≤ 25%, diffuse architecture, capsular and vascular invasion. There was no statistically significant difference in sex, age, or hormonal function between the patients with benign and malignant adrenocortical tumors (P > 0.05) as showed in Table (2).

Diagnostic Performance for the clinicopathological features in the studied cases:

Tumor size ≥ 6.5 cm was found to be the criterion with 80% sensitivity and 100% specificity in differentiating ACC from ACA. Adrenocortical tumors weighing more than 50 grams were classified as malignant, with a sensitivity of 90% and a specificity of 100%.

Malignant tumors showed higher frequency for the Weiss criteria. With 60% sensitivity and 100% specificity, Weiss scores > 5 showed a strong diagnostic efficacy for malignancy in our study. The most sensitive criteria were "mitotic rate >5/50 HPF" and atypical mitosis (100%), while the least sensitive criteria were lympho-vascular invasion, clear cells ≤ 25%, and diffuse architecture > 1/3 (35%, 45%, and 45%, respectively). Tumor size, weight, Weiss score, "mitotic rate >5/50 HPF," wide necrosis, positive lympho-vascular and capsular invasion- were the most specific criteria (100%), but the least specific criterion was the nuclear Fu¨hrman grade (65%) as shown in **Table (3).**

Immunohistochemical expression of IGF2, SMAD4 and PTTG1 among studied cases

In all control cases, IGF2 expression was negative. Just 5% of ACA cases and 80% of ACC cases had positive cytoplasmic staining in tumor cells, with a high statistically significant correlation in the differentiation between ACC& ACA (p<0.01) **(Table 4 & Fig. 1: A, B).** Ninety percent of the ACC cases that were studied showed no expression of SMAD4. As indicated in **Table (4)** and **Figure (1: C& D)-** there was a significant correlation between the expression of SMAD4 in the cytoplasm of tumor cells and the tumor type, being more expressed in ACA than ACC (P value <0.05). The PTTG1 was identified as brown nuclear staining in the tumor cells. It was positive in 75% of ACC cases. PTTG1 expression was higher among patients with ACC than ACA, (P value <0.01) as demonstrated in **Table (4) & Figure (1: E, F)**.

ROC curve analysis for the diagnostic performance of IGF2, SMAD4 and PTTG1 in differentiating ACC from ACA when used individually:

In terms of adrenocortical carcinoma differentiation when using the studied markers individually, IGF2 had the best specificity & sensitivity (95% & 80 % respectively). PTTG1 is the second one with 90% specificity & 75% sensitivity. However- as **Figure (2**) illustrates- SMAD4 exhibited a lower specificity and a higher sensitivity (60 & 90%, respectively).

Combined expression of the studied markers (IGF2, SMAD4 and PTTG1) in differentiating adrenocortical tumors:

The combined expression of IGF2, SMAD4, and PTTG1- had a high sensitivity of 100% in discriminating adrenocortical adenoma from carcinoma with 30% specificity as seen in **Table (5)**.

Correlation between IGF2, SMAD4 and PTTG1expressions in the studied cases:

The Spearman correlation analysis revealed a close relation between the expressions of IGF2, SMAD4, and PTTG1 in the adrenocortical tumor cases, with a statistically significant positive correlation between IGF2 and PTTG1 (r=.898). Furthermore, IGF2 and SMAD4 had a negative correlation in the studied cases (r=-0.313). However, there was insignificant statistical correlation

between SMAD4 and PTTG1 (r=- 0.207).

Table (1): IGF2, SMAD4 and PTTG1 antibodies in studied cases

Antibody	Type	Cat.No.	Dilution	Positive control	incubation	Antigen retrieval
IGF2	Mouse Monoclonal antibody	Thermo Fisher Scientific , Cat. No. MA5-17096	1:100	Urinary bladder cancer	15 minutes	Citrate buffer PH 6.0
SMAD4	Mouse Monoclonal antibody	Thermo Fisher Scientific, Cat. No. MA5-15682	1:100	Meningioma	30 minutes	Citrate buffer PH 6.0
PTTG1	Rabbit Polyclonal antibody	Thermo Fisher Scientific, Cat. No **34-1500**	1:50	Glioma	30 minutes	Citrate buffer PH 7.2

IGF2: Insulin growth factor 2, **SMAD4:** SMAD family member 4**, PTTG1**: Pituitary-tumor transforming gene

Table (2): Comparison of clinicopathological features among the studied ACA and ACC cases:

Clinicopathologic features		ACA	ACC	P value
Age (years)	**Mean**	**49.2**	**58.2**	0.32
	Range	**30-67**	**46-85**	
Sex	**Male**	**6 (30%)**	**12 (60%)**	0.05
	Female	**14 (70%)**	**8 (40%)**	
Tumor location	**Right**	**8 (40%)**	**11 (55%)**	0.04
	Left	**6 (30%)**	**9 (45%)**	
	Bilateral	**6 (30%)**	**0 (0%)**	
Hormonal function	**Cushing syndrome**	**10 (50%)**	**6 (30%)**	0.30
	Virillizing syndrome	**3 (15%)**	**1(5%)**	
	Non-functioning	**7 (85%)**	**13 (65%)**	
Tumor size (cm)	**Mean**	**2.9**	**11.8**	< 0.001**
	≥6.5 cm	**0**	**16**	
Tumor weight (g)	**Mean**	**6.2**	**510.1**	< 0.001**
	≥50 g	**0**	**18**	
Nuclear grade Fu¨rhman III/IV		**7**	**18**	< 0.001**
Mitotic rate >5/50 HPF		**0**	**20**	< 0.001**
Atypical mitosis		**1**	**14**	< 0.001**
Clear cells ≤ 25%		**2**	**9**	0.012**
Diffuse architecture > 1/3		**1**	**10**	0.001**
Necrosis		**0**	**18**	< 0.001**
Lymphovascular invasion		**0**	**7**	0.003**

Capsular invasion	**0**	**15**	< 0.001**
Total Weiss score (mean)	**0.50**	**5.8**	< 0.001**
Total	**20**	**20**	**-**

ACA: adrenocortical adenoma, ACC: adrenocortical carcinoma ,** Highly significant

Table (3): Diagnostic Performance for the clinicopathological features in the studied cases

	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
Nuclear Fuhrman grade	90.0	65.0	72.0	86.7	77.5	0.775
Mitotic rate >5/50 HPF	100	100	100	100	100	1.0
Atypical mitosis	100	100	100	100	100	1.0
Clear cells <25	45.0	90.0	81.8	62.1	67.5	0.675
Diffuse architecture >1/3	45.0	95.0	90.9	65.5	72.5	0.725
Necrosis	90	100	100	90.9	95.0	0.95
Venous invasion	35.0	100	100	60.6	67.5	0.675
Capsular invasion	75.0	100	100	80.0	87.5	0.875
Weiss score	60.0	100	100	71.4	80.0	0.80
Tumor Size	80.0	100	100	83.3	90.0	0.90
Tumor Weight	90.0	100	100	90.9	95.0	0.95

PPV: Positive Predictive Value **,NPV:**Negative Predictive Value **,AUC:** Area Under the curve

Table (4): Relation of immunohistochemical expression of IGF2, SMAD4 and PTTG1 in studied adrenocortical adenoma and carcinoma:

Studied groups	IGF2			SMAD4			PTTG1			Total
	-ve	**+ve**	**P**	**-ve**	**+ve**	**P**	**-ve**	**+ve**	**P**	
Adrenocrtical	**19**	**1**	****	**12**	**8**	*	**18**	**2**	****	**20**
adenoma	**(95%)**	**(5%)**	**0.00**	**(60%)**	**(40%)**	**0.02**	**(90%)**	**(10%)**	**0.00**	**(50%)**
Adrenocortical	**4**	**16**	**0**	**18**	**2**	**8**	**5**	**15**	**0**	**20**
carcinoma	**(20%)**	**(80%)**		**(90%)**	**(10%)**		**(25%)**	**(75%)**		**(50%)**
Total	**23**	**17**	**-**	**30**	**10**	**-**	**23**	**17**	**-**	**40**
	(57%)	**(43%)**		**(75%)**	**(25%)**		**(57%)**	**(43%)**		

IGF2: Insulin growth factor 2, **SMAD4:** SMAD family member 4**,**

PTTG1: Pituitary-tumor transforming gene **,*** significant, **highly significant

Table (5): Diagnostic Performance of combined expression of the studied markers IGF2, SMAD4 and PTTG1 in differentiating adrenocortical tumors:

	Sensitivity	**Specificity**	**PPV**	**NPV**	**Accuracy**	**AUC**
Combined markers	**100**	**30.0**	**58.8**	**100**	**65.0**	**0.65**

PPV: Positive Predictive Value **,NPV:**Negative Predictive Value **,AUC:** Area Under the curve

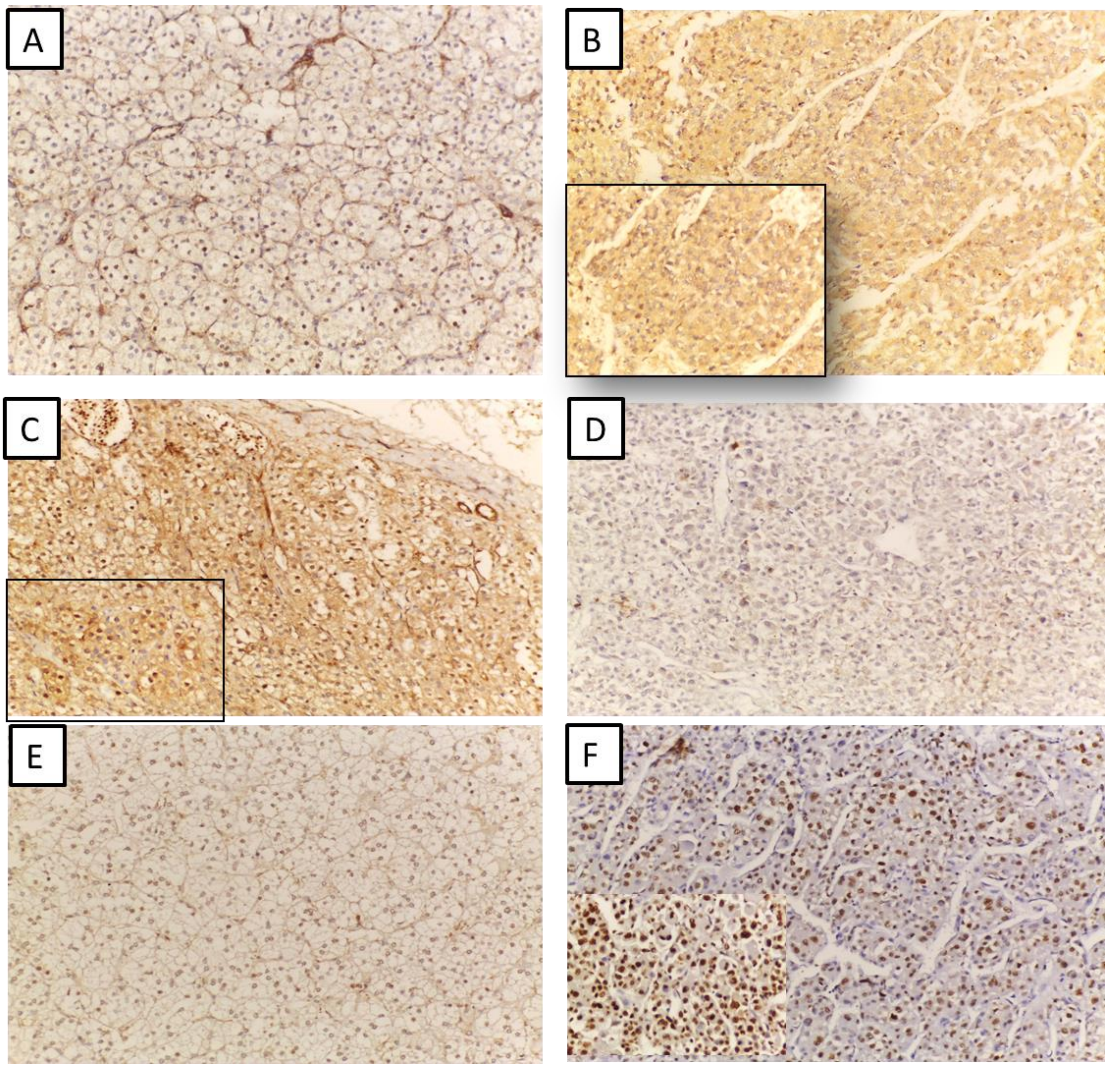

Figure (1): IGF2, SMAD4 and PTTG1 immunohistochemical expressions in adrenocortical adenoma (ACA) and adrenocortical carcinoma (ACC). (A): adrenocortical adenoma with negative expression for IGF2 while it showed strong diffuse cytoplasmic staining in adrenocortical carcinoma (B) (IHC, 200x), inset (IHC ,400x) .(C) Diffuse brown cytoplasmic expression of SMAD-4 in adrenocortical adenoma (IHC, 200x), inset (IHC ,400x). while (D) it was completely negative in adrenocortical carcinoma .(E) PTTG 1 with negative expression in adenoma and (F) higher nuclear positivity in carcinoma, (IHC, 200x), inset (IHC ,400x).

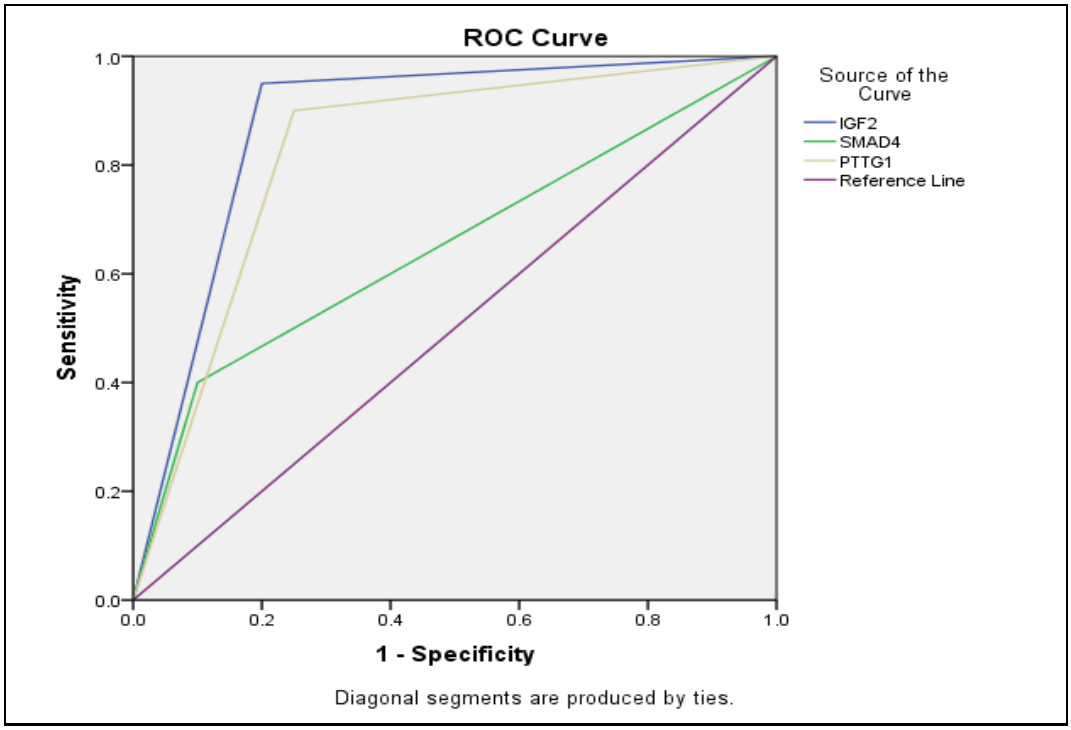

Figure (2): The diagnostic performance of IGF2, SMAD4 and PTTG1 in differentiating ACC from ACA**.**

Discussion

Adrenocortical carcinoma (ACC) is a highly aggressive rare tumor [23]. Proper diagnosis is essential, as the prognosis, follow-up, and treatment plans for ACC are significantly different from those of benign tumors. Thus, differentiating between malignant and benign adrenal cortical tumors presents a significant challenge for both clinicians and pathologists [24].

The Weiss classification system is commonly utilized to distinguish between benign and malignant adrenal cortical tumors. Despite its widespread use, its application is subjective and may not always be applicable. Differentiation between adrenal cortex cancer (ACC) and adenoma (ACA) remains a challenging task [25].

Our analysis of clinicopathological data revealed that tumor size, weight, and the Weiss system- were all effective clinicopathological criteria for distinguishing malignant from benign tumors (P < 0.001). This finding is in agreement with the results of a previous study [21].

The study revealed that tumors weighing ≥ 50 g were considered significant malignant tumors, with a 90% sensitivity rate and 100% specificity. Moreover, a threshold tumor size of ≥ 6.5 cm for identifying ACC demonstrated an 80% sensitivity rate and 100% specificity. These results were consistent with a previous studies [7, 26], which showed that tumor size had 100% sensitivity and 91.7% specificity in distinguishing adrenocortical carcinoma from adenoma and tumors with a weight of more than 50 grams that followed a malignant course.

The retrospective analysis evaluated the Weiss criteria for all 40 cases, demonstrating that a total Weiss score of 5 or higher displayed 60% sensitivity and 100% specificity in predicting malignancy. This conclusion aligns with a prior study [7], which examined the expression of the Weiss score as a highly sensitive parameter with 96% specificity and 100% sensitivity in malignant tumors.

The study at hand determined that the criteria with the highest sensitivity were 'mitotic rate >5/50 HPF' and atypical mitosis (100%), while the criteria with the lowest sensitivity were lympho-vascular invasion, clear cells ≤ 25%, and diffuse architecture > 1/3 (with sensitivities of 35%, 45%, and 45%, respectively). These findings align with those of a prior study [21], which identified 'mitotic rate >5/50 HPF' as the most sensitive criterion (100%), and '≤25% clear cells' or 'sinusoid invasion' as the least sensitive criteria (with sensitivities of 36%).

The most precise criteria were tumor size, weight, Weiss score, and mitotic rate greater than 5 per 50 HPF, as well as wide necrosis and positive lympho-vascular and capsular invasion (100%). The least particular criterion was the nuclear Fuhrman grade (65%). This is consistent with a previous study [21] that found the most particular criteria to be 'mitotic rate greater than 5 per 50 HPF,' 'necrosis,' 'venous invasion,' 'sinusoid invasion,' and 'capsular invasion' (100%), and the least particular was 'Fu¨hrman nuclear grade III/IV' (68%).

Moreover, our findings were consistent with those of a prior investigation [7], which demonstrated that a mitotic rate of ≥5 mitoses per 50 high-power fields examined through a ×40 objective lens was a strong indicator of malignancy, with a specificity of 100% and a sensitivity of 96%. To this point, numerous methods have been explored to differentiate between benign and malignant adrenocortical tumors, including genotyping techniques, molecular profiling studies, and immunohistochemical approaches [27]-

In the present study, insulin-like growth factor 2 (IGF2) exhibited negative expression in all control cases, whereas it displayed positive results in 80% of adrenocortical carcinoma (ACC) cases and only 5% of adrenocortical adenoma (ACA) cases- demonstrating a statistically significant difference between the two groups (P value <0.01). This finding aligns with previous research [24], which reported that 85% of ACC cases showed IGF-2 overexpression. These findings may provide valuable insights into the role of IGF2 in the pathogenesis and malignant transformation of ACA, supporting the adenoma-carcinoma sequence concept in adrenocortical tumors.

In comparison, a previous study conducted in 2006 [28] investigated IGF-2 immunohistochemistry in 17 ACCs and 22 ACAs- using different scoring systems and antibody clones. This discrepancy may be attributed to the variation in the methods employed.

IGF2 triggers the activation of tyrosine kinase receptors, which subsequently activate the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase

(PI3K)/Akt pathways. Activated Akt can initiate the activation of the mammalian target of rapamycin (mTOR) pathway. These pathways are implicated in the proliferation, survival, and metastasis of cancer cells [24].

By using ROC curve to determine the performance of IGF2 in detection of adrenocortical carcinoma, we found that IGF2 had the highest specificity and sensitivity (95%) and 80 %, respectively. This is in agreement with a previous study [5] that showed that ACC and benign lesions had a specificity of 95.5% and a sensitivity of 76.5%. Another study [12] reported a specificity and sensitivity of 100% for IGF2 in differentiating between ACA and ACC **[24]**.

SMAD4 expression was detected in the cytoplasm of tumor cells, and its level was closely related to the tumor type. It was found to be more highly expressed in the ACA than in the ACC ($P < 0.05$). In the current study, SMAD4 expression was significantly lower in ACCs than that in ACAs. This finding aligns with the results of a previous study [21]. This observation suggests that the downregulation of SMAD4 expression may contribute to the development of adrenocortical carcinoma and underscore its tumor-suppressive function.

The TGF-β/SMAD4 signaling pathway is known for its tumor-suppressive function during the initial stages of development, primarily through the induction of cell cycle arrest and apoptosis. SMAD4, which serves as the central mediator of TGF-β signaling, is often specifically inactivated in various types of cancers [14].

Various investigations conducted over the past two decades have disclosed that the loss of SMAD4 does not instigate the development of tumors, but can facilitate the progression of tumors initiated by other genetic factors, such as the activation of KRAS in pancreatic duct adenocarcinoma [29] and the inactivation of APC in colorectal cancer [30].

The results obtained from the ROC curve indicated that SMAD4 exhibited reduced specificity (60%) and enhanced sensitivity (90%) for distinguishing ACA from ACC. This finding aligns with a previous study [21], which reported that negative/low expression of SMAD4 was highly sensitive in differentiating ACCs from ACAs (sensitivity = 92%), yet displayed a low specificity of merely 40%.

The immune-expression of PTTG1 was detected by brown nuclear staining of the tumor cells. It was positive in 75% of ACC cases, with higher expression among patients with ACC than in those with ACA ($P < 0.01$), in agreement with the prior study [22].This finding is supported by the fact that PTTG1 acts as an oncogene, and that high expression of PTTG1 can promote tumorigenesis in many types of tumors, such as prostate cancer [17] and bladder tumors [31].

PTTG1 encodes a protein that suppresses the transcriptional activity of p53 and p53 mediated apoptosis. Securin is involved in G2/M transition, regulating the transition into M phase

and sister chromatid separation [32]. Furthermore, PTTG1 serves as a transcription activator of the c-myc oncogene and is involved in various molecular mechanisms such as epithelial-mesenchymal transition (EMT), PI3K/AKT signaling, and mitogen-activated protein kinase (MAPK) signaling pathways [33].

Upon testing the performance of the ROC curve in this study, PTTG1 was found to be the second best- after IGF2- in differentiating adrenocortical carcinoma, with 90% specificity and 75% sensitivity.

The present study showed that IGF2 expression was higher in patients with a Weiss score of >5 (P <0.05). It did not show any statistical relationship with other clinicopathological variables (P <0.05), which is consistent with a previous work [21]. However, there was no statistically significant relationship between SMAD4 or PTTG1 expression and other clinicopathological parameters (P <0.05), which is consistent with a previous study [21].

The current study revealed an inverse relationship between IGF2 and SMAD4 expression (r= 0.313). The increase in IGF2 and decrease in SMAD4 may play an autocrine/paracrine role in adrenal tumorigenesis and contribute to malignant progression, which may be explained by the suppression of SMAD4 expression in pancreatic cancer by miR483-3p, which is located in intron 2 of IGF2. The immunohistochemical staining for SMAD4 labelled as ‘negative/low’ indicated its deregulation, and was associated with overexpression of miR483-3p and IGF2 [34].

Furthermore, the current study found a statistically significant positive correlation between IGF2 and PTTG1 in adrenocortical tumors (r= 0.898). They have a synergistic effect on the malignant progression of adrenocortical tumors. However, there was an insignificant statistical correlation between SMAD4 and PTTG1 (r=-0.207).

Conclusion:

IGF-2, SMAD4, and PTTG1- may be used as reliable and promising diagnostic biomarkers for malignancy in adrenal cortical neoplasms, which may also have therapeutic implications. Both IGF2 and PTTG1 may have synergistic actions in the malignant progression of adrenocortical tumors.

The combined expression of this immunohistochemical panel of markers (IGF2, SMAD4, and PTTG1) and variable clinicopathological parameters (tumor size, weight, and mitotic rate >5/50 HPF)- plays a significant role in differentiating adrenocortical adenoma from carcinoma.

References

1- **Fassnacht M, Assie G, Baudin E, Eisenhofer G, De La Fouchardiere C, Haak HR, et al .** Adrenocortical carcinomas and malignant phaeochromocytomas: ESMO–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology. 2020 Nov 1;31(11):1476-90.

2- **Sherlock M, Scarsbrook A, Abbas A, Fraser S, Limumpornpetch P, Dineen**

R, et al . Adrenal incidentaloma. Endocrine reviews. 2020 Dec 1;41(6):775-820.

3- Kerkhofs TM, Verhoeven RH, Van der Zwan JM, Dieleman J, Kerstens MN, Links TP, et al . Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993. European Journal of Cancer. 2013 Jul 1;49(11):2579-86.

4- Mokhtar N, Salama A, Badawy O, Khorshed E, Mohamed G, Ibrahim M, et al . Cancer pathology registry 2000-2011. Cairo, Egypt: National Cancer Institute Cairo University. Back to cited text. 2016;18.

5- Viëtor CL, Creemers SG, van Kemenade FJ, van Ginhoven TM, Hofland LJ, Feelders RA. How to differentiate benign from malignant adrenocortical tumors?. Cancers. 2021 Aug 30;13(17):4383.

6- Lau SK, Weiss LM. The Weiss system for evaluating adrenocortical neoplasms: 25 years later. Human pathology. 2009 Jun 1;40(6):757-68.

7- Aubert S, Wacrenier A, Leroy X, Devos P, Carnaille B, Proye C, et al. Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. The American journal of surgical pathology. 2002 Dec 1;26(12):1612-9.

8- Pohlink C, Tannapfel A, Eichfeld U, Schmidt F, Führer D, Paschke R, et al . Does tumor heterogeneity limit the use of the Weiss criteria in the evaluation of adrenocortical tumors?. Journal of Endocrinological Investigation. 2004 Jun;27:565-9.

9- Ahmed AA, Thomas AJ, Ganeshan DM, Blair KJ, Lall C, Lee JT, et al . Adrenal cortical carcinoma: pathology, genomics, prognosis, imaging features, and mimics with impact on management. Abdominal Radiology. 2020 Apr;45:945-63.

10- Crona J, Beuschlein F. Adrenocortical carcinoma—towards genomics guided clinical care. Nature Reviews Endocrinology. 2019 Sep;15(9):548-60.

11- Iams WT, Lovly CM. Molecular pathways: clinical applications and future direction of insulin-like growth factor-1 receptor pathway blockade. Clinical Cancer Research. 2015 Oct 1;21(19):4270-7.

12- Pereira SS, Monteiro MP, Costa MM, Moreira Â, Alves MG, Oliveira PF, et al . IGF2 role in adrenocortical carcinoma biology. Endocrine. 2019 Nov;66:326-37.

13- Dardare J, Witz A, Merlin JL, Gilson P, Harlé A. SMAD4 and the TGFβ pathway in patients with pancreatic ductal adenocarcinoma. International journal of molecular sciences. 2020 May 16;21(10):3534.

14- Zhao M, Mishra L, Deng CX. The role of TGF-β/SMAD4 signaling in cancer. International journal of biological sciences. 2018;14(2):111.

15- Demeure MJ, Coan KE, Grant CS, Komorowski RA, Stephan E, Sinari S, Mount D, et al. PTTG1 overexpression in adrenocortical cancer is associated with poor survival and represents a potential therapeutic target. Surgery. 2013 Dec 1;154(6):1405-16.

16- Xiang W, Wu X, Huang C, Wang M, Zhao X, Luo G, et al . PTTG1 regulated by miR-146a-3p promotes bladder cancer migration, invasion, metastasis and growth. Oncotarget. 2017 Jan 1;8(1):664.

17- Fraune C, Yehorov S, Luebke AM, Steurer S, Hube-Magg C, Büscheck F, et al. Upregulation of PTTG1 is associated with poor prognosis in prostate cancer. Pathology International. 2020 Jul;70(7):441-51.

18- Mete O, Erickson LA, Juhlin CC, de Krijger RR, Sasano H, Volante M, et al. Overview of the 2022 WHO classification of adrenal cortical tumors. Endocrine pathology. 2022 Mar;33(1):155-96.

19- Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA: a cancer journal for clinicians. 2017 Mar;67(2):93-9.

20- Guntiboina VA, Sengupta M, Islam N, Barman S, Biswas SK, Chatterjee U, et al . Diagnostic and prognostic utility of SF1, IGF2 and p57 immunoexpression in

pediatric adrenal cortical tumors. Journal of Pediatric Surgery. 2019 Sep 1;54(9):1906-12.

21- Wang C, Sun Y, Wu H, Zhao D, Chen J. Distinguishing adrenal cortical carcinomas and adenomas: a study of clinicopathological features and biomarkers. Histopathology. 2014 Mar;64(4):567-76.

22- Romero Arenas MA, Whitsett TG, Aronova A, Henderson SA, LoBello J, Habra MA, et al. Protein expression of PTTG1 as a diagnostic biomarker in adrenocortical carcinoma. Annals of surgical oncology. 2018 Mar;25:801-7.

23- Scollo C, Russo M, Trovato MA, Sambataro D, Giuffrida D, Manusia M, et al. Prognostic factors for adrenocortical carcinoma outcomes. Frontiers in Endocrinology. 2016 Jul 25;7:99.

24- Mizdrak M, Tičinović Kurir T, Božić J. The role of biomarkers in adrenocortical carcinoma: a review of current evidence and future perspectives. Biomedicines. 2021 Feb 10;9(2):174.

25- Babińska A, Pęksa R, Wiśniewski P, Świątkowska-Stodulska R, Sworczak K. Diagnostic and prognostic role of SF1, IGF2, Ki67, p53, adiponectin, and leptin receptors in human adrenal cortical tumors. Journal of surgical oncology. 2017 Sep;116(3):427-33.

26- Sasano H, Suzuki T, Moriya T. Discerning malignancy in resected adrenocortical neoplasms. Endocrine Pathology. 2001 Dec;12:397-406.

27- Mete O, Asa SL, Giordano TJ, Papotti M, Sasano H, Volante M. Immunohistochemical biomarkers of adrenal cortical neoplasms. Endocrine pathology. 2018 Jun;29:137-49.

28- Schmitt A, Saremaslani P, Schmid S, Rousson V, Montani M, Schmid DM, et al . IGFII and MIB1 immunohistochemistry is helpful for the differentiation of benign from malignant adrenocortical tumours. Histopathology. 2006 Sep;49(3):298-307.

29- Ahmed S, Bradshaw AD, Gera S, Dewan MZ, Xu R. The TGF-β/Smad4 signaling pathway in pancreatic carcinogenesis and its clinical significance. Journal of clinical medicine. 2017 Jan 5;6(1):5.

30- Itatani Y, Kawada K, Sakai Y. Transforming growth factor-β signaling pathway in colorectal cancer and its tumor microenvironment. International journal of molecular sciences. 2019 Nov 20;20(23):5822.

31- Gong S, Wu C, Duan Y, Tang J, Wu P. A comprehensive pan-cancer analysis for pituitary tumor-transforming gene 1. Frontiers in Genetics. 2022 Feb 25;13:843579.

32- Bernal JA, Luna R, Espina Á, Lázaro I, Ramos-Morales F, Romero F, et al . Human securin interacts with p53 and modulates p53-mediated transcriptional activity and apoptosis. Nature genetics. 2002 Oct;32(2):306-11.

33- Cui L, Ren T, Zhao H, Chen S, Zheng M, Gao X, et al . Suppression of PTTG1 inhibits cell angiogenesis, migration and invasion in glioma cells. Medical Oncology. 2020 Aug;37:1-1.

34- Wilentz RE, Su GH, Le Dai J, Sparks AB, Argani P, Sohn TA, et al . Immunohistochemical labeling for dpc4 mirrors genetic status in pancreatic adenocarcinomas: a new marker of DPC4 inactivation. The American journal of pathology. 2000 Jan 1;156(1):37-43.

To cite this article: Omneya Y. Bassyoni, Ebtehal M. Abdel-Aal. Diagnostic Significance of Expression of IGF2, SMAD4, PTTG1 and Clinicopathological Parameters in Differentiating Adrenocortical Tumors. BMFJ 2024;41(5):372-385