Evaluating diagnostic and prognostic significance of steroidogenic factor-1(SF-1) and gherlin in differentiation between adrenocortical adenoma, adrenocortical carcinoma and pheochromocytoma

(immunohistochemical study)

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Background: Adrenal masses are a common finding in the adrenal gland. Steroidogenic factor 1 (SF1) is a nuclear transcription factor that regulates genes involved in gonadal and adrenal steroidogenesis. Ghrelin is a ligand of growth hormone secretagogue receptor type 1 (GHS-R1). The principal role of ghrelin is to regulate energy homeostasis and secretion of growth hormone.

Aim: to differentiate adrenocortical adenoma, carcinoma and pheochromocytoma immunohistochemically using stroidogenic factor-1 (SF-1) and gherlin expression and evaluate their diagnostic and prognostic significance in relation to various clinicopathologic parameters.

Material and methods: This is retrospective study carried upon 45 of different cases of adrenal gland lesions designated as: 20 cases of adrenocortical adenomas(ACA) and 10 cases of adrenocortical carcinomas(ACC) and 15 cases of pheochromocytoma . Immunohistochemical staining techniques were used to detect the role of SF-1 and gherlin in the forementioned adrenal gland lesions and evaluate their relations to different clinicopathological data and patient's survival.

Results: SF-1 and gherlin were sensitive (100%) and specific (95%, 100% respectively) immunohistochemical markers in diagnosis adrenocortical carcinoma, but gherlin was more sensitive (100%) and SF-1 was more specific (60%) in diagnosing adrenocortical adenoma. On the other side, SF-1 was more sensitive(100%) in diagnosing pheochromocytoma. SF-1 expression in adrenocortical carcinoma revealed that strong positive SF-1expression was associated with reduced overall survival .

Conclusion:. SF1 and ghrelin immunoreactivity may be considered sensitive and specific markers for differentiating ACC from ACA and pheochromocytoma.

However, further research is required to determine the causes of differential ghrelin expression in adrenal tumors.

Key words: SF-1 ; gherlin ; ACA; ACC ; pheochromocytoma.

INTRODUCTION

Adrenal neoplasms are a common finding that are discovered in the adrenal gland. However, a major counterpart of these neoplasms are benign, cancer of the adrenal gland should be considered when the symptom of the patient is hypercortisolism with the adrenal mass [(1)].

Primary adrenal tumors compose two main different types. Adrenal tumors that are derived from the adrenal cortex and mainly include adrenal cortical adenoma and cortical carcinoma. Adrenal tumors that originate from the adrenal medulla contain pheochromocytoma (PCC) and neuroblastic tumors (NT) [(2)].

The prevalence of the adrenal tumors is nearly 1.4% but 1.9% to 3.2% in those older than 65 years. Most of cases are benign adenomas and some lesions are diagnosed rarely such as pheochromocytoma and adrenocortical carcinoma (ACC) [(3)]. However most of these neoplasms are nonfunctioning with benign behavior and some of these neoplasms including functioning tumors pheochromocytoma and adrenal cortical carcinoma can affect health of the patients significantly and therapeutic interventions are required to deal with these neoplasms [(4).]

Adenomas of the adrenal gland are benign neoplasms and its origin is the cortex of the adrenal gland. Majority of adenomas of the adrenal gland are incidentally discovered during imaging of the abdomen for another reason , so, it is called “adrenal incidentaloma.” The importance of these adrenal adenomas comes from the ability of these neoplasm to secrete hormones. Although many adrenal adenomas are non-functioning, these adenomas can overproduce hormones of the adrenal cortex [(5)].

Adrenocortical carcinomas (ACC) are very rare neoplasm. The incidence of ACC globally is half to two per one million people yearly. Adrenocortical carcinoma is a neoplasm that has an aggressive behavior and some of these neoplasms are functioning and the patient presents with virilization and/or Cushing syndrome. In

most cases, patients with ACC present with mass of the abdomen or discovered incidentally and mostly of these neoplasms are non-functioning. It represents 0.02 to 0.2% of all cancer-related deaths [1].

In Egypt, the benign tumors of adrenal gland comprises 9.01% of all suprarenal neoplasms and adrenocortical adenoma comprises 40.48% of the benign adrenal gland tumors**,** while primary malignant adrenal neoplasms represented 0.67% of all malignancy at National Cancer Institute(NCI), adrenocortical carcinoma represented 11.15% and pheochromocytoma represents 23.8% of adrenal neoplasms [6].

Recently, there is dramatic increase in the incidence of adrenal masses in recent years because of increasing the use of improved and recent imaging techniques. Evaluation of the adrenal masses that are discovered incidentally requires a multidisciplinary team to detect the proper diagnosis , intervention and also the prognosis of these cases [7].

Steroidogenic factor 1 (SF1) is one of the steroid receptor superfamily. It is a nuclear transcription factor that regulates genes involved in gonadal and adrenal steroidogenesis. It is expressed within steroidogenic tissues of the gonads, adrenal glands and the anterior pituitary gland . SF1 may be useful in resolving difficult differential diagnoses for tumors emerging from the adrenal gland and gonads [8].

Ghrelin is considered a growth hormone secretagogue receptor type 1 (GHS-R1)'s ligand and it has receptors which are GHS-R1a and GHS-R1b and these receptors were discovered in the adrenal gland of human. Gherlin has been found to have a 28-amino acid peptide and having in serine 3 position an n-octanoyl moiety . During post-translational cleavage, ghrelin is produced from the preproghrelin precursor [9] . It is secreted originally by endocrine cells of the gastric mucosa, but it is also expressed in many other organs, including the small and large intestine, pituitary, hypothalamus, testes, ovaries and adrenal glands. Ghrelin has a main role in secretion of growth hormone and regulating energy homeostasis. Ghrelin affects various organs physiologically and pathologically and many researches have confirmed these effects [2] .

The aim of this work is to differentiate adrenocortical adenoma, carcinoma and pheochromocytoma immunohistochemically using stroidogenic factor-1 (SF-1) and gherlin expression and evaluate their diagnostic and prognostic significance in relation to various clinicopathologic parameters.

MATERIAL &METHODS:

This was a retrospective , selected study comprising of 45 different cases of adrenal gland lesions designated as: 20 cases of adrenocortical adenomas(ACA) and 10 cases of adrenocortical carcinomas(ACC) and 15 cases of pheochromocytoma, over the last 10 years from June 2012 to May 2022 cases. The cases were collected from Pathology Department, Early Cancer Detection Unit, Faculty of Medicine, Benha University, Egypt.

Inclusion criteria: Cases with available clinicopathological data regarding; age, sex, laterality, tumor size, tumor weight, primary tumor (T), grade, lymph node status, distant metastasis and stage.

Exclusion criteria: Cases with no available paraffin blocks or clinicopathological data- were excluded from the current study.

. The Ethics Committee of Faculty of Medicine, Benha University, Egypt approved this study code{ **RC 5-8-2024**}.

Histopathological Analysis:

Formalin fixed /Paraffin embedded blocks were cut at 5 µm thickness and stained using hematoxylin and eosin stain. Two observers reviewed the microscopic sections from all the cases. The Weiss criteria was used to classify adenomas and carcinomas (Weiss score ≥ 3 indicates a diagnosis of ACC) [10] .The grading system uses the cut-off of 20 mitoses per 10 mm2 to distinguish low- and high-grade ACC. Low-grade ACC has a mitotic activity ≤ 20 mitoses per 10 mm2, whereas high-grade ACC shows > 20 mitoses per 10 mm2 [11] . PASS score and the GAPP - were used to assess metastatic potential of pheochromocytoma (PASS ≤4 : considered for malignancy([12] . Lymph node status was evaluated and ENSAT staging system was applied to the ACC cases according to AJCC, 8th edition[13] .

Immunohistochemical study:

On positive charge slides, three to four mm thick paraffin-embedded tissue sections which were formalin-fixed- were prepared. For immunohistochemical analysis, the streptavidin-biotin technique is utilized in compliance with the manufacturer's guidelines. Antibodies are shown in **Table (1).**

For the secondary developing reagents, we used a standard labeled streptavidin-biotin system *(**Dako Catalysed Signal Amplification System, Peroxidase, K1500; Dako, Copenhagen, Denmark).***

The prepared sections were stained with a 0.02% diluted solution of diamethoxybenzidine. After that, hematoxylin was used as a counterstain. The primary antibody stage was omitted for each marker, and the normal rabbit serum IgG was used as a negative control in its stead.

SF-1 interpretation:

SF-1 was detected as nuclear brown coloration. Immuno reactivity was evaluated qualitatively into a three-tier system: 1 + when nuclear positivity was seen in less than 50% of the tumor; 2 + when 50% or more but less than 80% of the tumor .section showed positivity for the marker; 3 + when nuclear positivity was seen in 80% or more of the tumor section [(14).]

Ghrelin interpretation:

Ghrelin was detected as cytoplasmic brown coloration The intensity of the staining in the tumor cells was examined and scored on a scale as non-immunoreactive (non-IR, 0), weak (1), moderate (2) and strong (3), respectively[(15).]

STATISTICAL ANALYSIS:- The collected data was recorded then presented, and statistically analyzed by computer using Statistical Package for the Social Sciences (SPSS) 25.0 for windows (SPSS Inc., Chicago, IL, USA).

Categorical data were expressed as numbers and percentages. Numerical data were expressed as mean ± standard deviation. Pearson Chi square test(X2) was used to assess relations between groups. Survival analysis and Kaplan Meier curve- were

performed for carcinoma cases. P-value >0.05 was considered non-significant (NS), <0.05 significant (S), ≤ 0.01 highly significant (HS).

RESULTS:

CLINICOPATHOLOGICAL RESULTS:

This study was carried out upon 45 cases of Adrenal gland lesions. The age of studied cases ranged from 19 years to 75 years with mean age (49.64±14.57) years,. The mean size of ACA cases was 4.7±1.8 cm (range 3-11cm), and the mean size of ACC cases was 14±2.8cm (range 10-20cm). the mean size of pheochromocytoma 5.5±2.6 (range 2-11). The weight of ACA cases ranged from 14gm up to 50 gm in the largest dimension, with mean weight 30.5±10.1. The ACC cases ranged from 230 gm to -995 gm, with mean weight 412.9±218.9. The pheochromocytoma cases ranged from 30 gm up to 1650gm, with mean weight 242.4±409.9 .

Immunohistochemical results:

SF-1 expression

SF-1 was detected as brownish nuclear staining. In ACA most cases were negative 18(90%) while 2 (10%) cases were positive with a grade of (+1) expression. whereas in ACC ,all cases were positive with a grade of +3 in 7 (70%) of cases and a grade (+2) in 3 cases (30%). On the other side all cases of pheochromocytoma were negative, **Table(2).**

A highly statistically significant relation was found between SF-1 expression and studied cases (P =>.0001).

Statistical analysis was performed on relation between SF-1 expression in studied Adrenocortical Carcinoma cases and clinicopathological variables.

A statistically significant relation was found between SF-1 expression and Lympho-vascular invasion, Grade, Necrosis, nuclear grade**,** primary tumor ,lymph node metastasis ,distant metastasis and stage of the studied adrenocortical carcinoma cases (P value >0.05), **Table(3).**

Ghelin expression

Gherlin was detected as brownish cytoplasmic staining. In ACA, all cases were positive with the most prevalent grade was grade 1(60%) while grade 2 in (30%). Whereas in ACC ,all cases were negative (100%). On the other side, all cases of pheochromocytoma were positive with the most prevalent grade was grade 3(66.6%).

A highly significant statistical relation was found between gherlin expression and studied cases (P =>.0001), **Table(4).**

Statistical analysis was performed on relation between Gherlin expression in studied adrenocortical adenoma cases and clinicopathological variables and revealed no statistical significant relation between them.

Statistical analysis was performed on relation between Gherlin expression in studied pheochromocytoma cases and clinicopathological variables.

Statistical significant relation between gherlin expression and cellular spindling and nuclear pleomorphism, **Table(5).**

The diagnostic accuracy of both markers expression for differentiating between ACA, ACC and pheochromocytoma- was determined by using ROC Curve. The curve shows the specificity (true negative fraction) and sensitivity (true positive fraction) of the test. The area under the curve indicates the test's accuracy (AUC) **(Figure 1)**. ACA: SF-1(sensitivity 10%., specificity 60%, AUC .330), Gherlin (sensitivity 100%, specificity 40% , AUC .506). ACC: SF-1(sensitivity 100%., specificity 95%, AUC 1.00), Gherlin (sensitivity 100%, specificity 100%, AUC 1.00). Pheochromocytoma, SF-1 (sensitivity 100%., specificity 40%,AUC .700) ,Gherlin (sensitivity 86.7%,specificity 74.3% , AUC.882).

So, both markers were sensitive and specific in diagnosis of adrenocortical carcinoma, but gherlin was more sensitive and SF-1 was more specific in diagnosing adrenocortical adenoma. On the other side, SF-1 was more sensitive(100%) but Gherline was more specific(74.3%) in diagnosing pheochromocytoma.

Relation between SF-1and over all survival &recurrence

A Kaplan-Meier survival analysis performed for SF-1 expression in adrenocortical carcinoma revealed that ,strong positive SF-1expression was associated with reduced

overall survival (P 0.027)**.** There was no association between SF-1 and recurrence (P 0.48)**, Figure (2).**

There was a highly significant negative correlation between SF-1 expression and gherlin expression in adrenal lesions (p <.001 (HS)) , **Table (6).**

DISCUSSION:

Adrenal masses are frequent tumors and adrenocortical adenoma are considered the most common of these adrenal masses. Adrenal cortical carcinoma is a rare tumor and the most common primary malignancy arising from adrenal gland [(16)]. Pheochromocytomas are infrequent tumors arisig from adrenal medulla [(17)].

Adrenal incidentalomas (AI) are incidentally diagnosed adrenal gland neoplasms that are discovered through radiologic examinations for different aims. The major counterpart of these tumors when discovered and diagnosed are adrenocotical adenomas and accounts approximately 80% of AI, while adrenocortical carcinoma and pheochromocytoma are uncommon neoplasms and discovered incidentally at a rate of 5% of adrenal incidentalomas (AI) [(18)].

The main problematic issue is to diffrentiate adrenocortical carcinoma from its diffrential diagnosis including pheochromocytoma and adrenocortcal adenoma. [(19)].

Steroidogenic factor 1 (SF-1) is an important nuclear transcription factor that plays a vital role in the development of steroidogenic tissues and regulation of steroid biosynthesis in the adrenal cortex [(20)].

The current study showed that SF1 expression was detected in all cases of ACCs (100%) including (70%) of these cases showed high SF-1 expression (grade 3). In contrast, the majority of adrenocortical adenoma cases (90%) showed negative SF-1 expression, while all cases of pheochromocytoma were negative for SF-1 expression **(P =0.000).** These results came in agreement with **Maity et al study [(20)]** that revealed positive expression of SF-1 in all cases of ACCs (100%), with a grade (3) in each case, while it showed only positive expression in 20% of cases of ACAs with grade (2) and negative expression in (80%) with a grade (1).

These findings came partly in agreement with the results of **Siberia et al** [21] study which detected strong SF-1 expression in 98% of cases of ACCs, negative SF-1 expression in pheochromocytoma cases, but, against the result of the current study concerning adrenocortical adenoma cases as whose study revealed high SF-1 expression in all benign adrenocortical tumors. This different finding in our study could be due to small number of our study group and genetic differences because of the different ethnicity .

The result of our study came also in agreememt with the result of **Babińska et al** [22] study which reported that SF-1 expression was associated with diagnosis of ACC.

These findings suggested that SF1 may have a pivotal role in development of adrenal gland and its function, from its incorporation in adrenal cortex formation to tumorigenesis and progression from adenoma to carcinoma. This suggestion proofed by reduced expression of SF-1 could affect appropriate adrenal organogenesis and its function [23].

The present study revealed a significant statistical relation between IHC expression of SF1 and grade (P =0.03), nuclear grade (P =0.01), stage (P =0.03), necrosis (P =0.01), and lympho-vascular invasion ((P =0.03). These findings explained by **Torti et al** [1] study which reported that SF-1 has a role in both fetal development of the adrenal cortex and steroidogenesis irrespective the steroidogenic process. The role of SF-1 in the molecular pathogenesis of ACC can be explained by detecting its cellular role in adrenocortical adenomas versus its role in ACC. It had been discovered that in ACC, it followed a phenotypical pattern that was similar to fetal development irrespectively the steroidogenic process, so, the profile of the tumors cellular showed less differentiation and didn't not match the gland's hormonal activity. While in adenomas the cellular profile of expression of SF-1 was following the pattern that was related to the steroidogenic process. All these findings gave a base to proof that increased expression of SF-1 was asociated with poor prognosis [24].

A Kaplan-Meier survival analysis revealed that expression of SF-1 in adrenocortical carcinoma was strong positive expression and was associated with reduced overall survival (P 0.027), but there was no association between SF-1 and recurrence (P 0.48)**.** These findings came partly in line with results of **Sbiera et al** [21]

study that revealed high SF-1 expression was associated with worse overall survival and associated with recurrence which diagreed our result in this point. The studies of **Babińska et al** [22] **and Torti et al** [1] also came in agrrement with our present study, as their studies reported that high expression of SF-1 in ACC was associated with reduced overall suvival.

Ghrelin is a small peptide associated with 28 amino acids and is considered a ligand of growth hormone secretagogue receptor type 1 (GHS-R1). Ghrelin has multifunctional roles as it plays a role in gastric acid release, insulin secretion, appetite, gastric motility, the turnover of gastric and intestinal mucosa regulation of energy balance. Expression of gherlin and its receptors farly from normal tissues suggests that it has an important role in regulating several processes related to cancer progression, especially in metastasis and proliferation other than its physiological function [22].

The current study showed that all of ACA cases (100%) and pheochromocytoma cases showed positive cytoplasmic expression of gherlin and all (100) of ACC cases showed negative expression of gherlin , with a statistically significant difference (P =0.000).

These findings were compatible with **Komarowska et al.2021**[25] study that reported that expression of ghrelin was significantly reduced in adrenocortical adenocarcinoma in relation to the ACA and pheochromocytoma cases.

Also, this results was in line with **Barzon et al** [26] study that demonstrated higher ghrelin expression level in all types of benign adrenal tumours (n = 34) and unchanged or slightly decreased expression in adrenal cancers (n = 6) compared to the healthy glands (n = 14).

The result of **Komarowska et al .2018**[27] study disagreed our result, as the result showed that highest expression of ghrelin was observed in carcinoma cases and the lowest in the control group. Expression of gherlin was 21 times higher in carcinoma and 2.4 times higher in adenoma compared with controls. These differences in the results might be due to different size of samples and different maneuver of samples analysis.

Several explanations could be suggested to explain ghrelin's low expression in ACC. Firstly, a gene mutation of ghrelin protein causes disturbances in its expression. When it is suggested that high ghrelin's immunoreactivity may be due to the presence of its binding sites (i.e. ghrelin receptors) in tissues that are examined, therefore, the decreased immunoreactivity may be linked to the decreased number of receptors — negative feedback-based defense down-regulation. Moreover, ghrelin plays a role in proliferation of adrenocortical cells as it acts a stimulatory factor. So, it has been suggested that ghrelin that is produced locally may stimulate auto or paracrine way to stimulate adrenal tumor cell proliferation . But, in ACC the low expression of ghrelin suggests that this mechanism is out of the way [26].

Out of pheochromocytoma cases of the current study, ten cases (66.6%) showed high gherlin expression (score 3), this finding can be explained by **Raghay et al [28]** study that demonstrated that gherlin was expressed in the medulla of the human or rat adrenal gland.

In this present study, there was a significant statistical relation between IHC expression of ghrelin and cellular spindling (P =0.01), and nuclear pleomorphism (P =0.03), in pheochromocytoma cases.

Our findings were compatible with **Raghay et al** [28] that studied its expression in the adrenal medulla and pheochromocytoma and reported that ghrelin expression was associated with poor prognostic factors as high grade and pleomorphism.

Using ROC curve analysis, both markers were sensitive and specific in diagnosis of adrenocortical carcinoma, but ghrelin was more sensitive, and SF-1 was more specific in diagnosing adrenocortical adenoma. On the other side, SF-1 was more sensitive (100%) but Ghrelin was more specific (74.3%)- in diagnosing pheochromocytoma.

This results were in line with study of **Babińska et al** [22] that showed that SF1 was sensitive and specific in diagnosis of adrenocortical carcinoma, and **Komarowska et al.2021** [25] study which suggested that ghrelin may serve as an important immunohistochemical marker of ACC due to its high specificity and sensitivity .

The diagnosis of ACC may be very difficult, this point should be taken into consideration during the histopathological examination. Therefore, immunostaining with gherlin may be a useful prognostic immunohistochemical marker for distinguishing aggressive and malignant ACC from benign ACA and pheochromocytoma.

There was a highly significant negative correlation between SF-1 expression and gherlin expression in adrenal lesions. This finding could be explained by gherlin can affect indirectly SF-1 expression in adrenal gland. As **Kinyua et al** [29] study found that adrenal steroidogenesis was regulated by insulin through increasing the activity and the expression of steroidogenic factor 1 (SF-1) both in vivo and vitro. Moreover, several updated studies on gherlin had afflicted understanding its mechanisms as it can cause metabolic dysfunctions as insulin resistance [30]. Therefore, we can conclude that increased gherlin expression in adrenal lesions can cause inulin resistance and then decreases expression of SF-1 and decreases the adrenal steroidogenesis.

Our research suggested that the expression profiles of SF-1 and ghrelin may be associated with the type of adrenal gland neoplasm. SF1 and ghrelin immunostaining may be considered as specific and sensitive markers for differentiating ACC from ACA and pheochromocytoma. However, further studies and researches are required to detect the causes and explanations of SF-1 and gherlin immunohistochemical expressions in adrenal gland neoplams.

Conflicts of interest: No conflicts of interest.

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Table(1):Antibodies used in the study

Antibody	**Source**	**Working concentration**	**Incubation period**	**Antigen retrieval**	**Positive control**
SF-1	***(PerseusProteomics, Tokyo, Japan)***	1:100	overnight at room temperature	Citric acid PH(6)	Colonic carcinoma
Ghrelin	***(ab57222; Abcam)***	1:100	Overnight at 4 C	Citric acid PH(6.1)	normal gastric mucosa

Table(2):correlation between score of SF-1 expression and study group

lesion N.=45	Score of SF 1 Expression				FET	P value
	0 N.=33 N. %	1 N.=2 N. %	2 N.=3 N. %	3 N.=7 N. %		
Adrenocortical adenoma(20)	18 90%	2 10%	0 0.0%	0 0.0%	47.0	.000(HS)
Adrenocortical Carcinoma (10)	0 0.0%	0 0.0%	3 30%	7 70%		

Pheochromocytoma(15)	15 100%	0 0.0%	0 0.0%	0 0.0%		

FET:fisher exact test HS:highly significant

Table(3) :Relation between score of SF-1 expression and Adrenocortical Carcinoma

Carcinoma N.=10		Score of SF 1 Expression		FET	P value
		2 N.=3 N. %	3 N.=7 N. %		
Age	≤50 (2)	0 0.0%	2 100.0%	1.07	.301
	>50(8)	3 37.5%	5 62.5%		
Sex	Female (3)	2 66.7%	1 33.3%	2.74	.09
	Male (7)	1 14.3%	6 85.7%		
Size	≤14 cm(6)	2 33.3%	4 66.7%	.079	.778
	>14 cm (4)	1 25.0%	3 75.0%		
Weight	≤370 gm (5)	1 20.0%	4 80.0%	.47	.49
	>370 gm (5)	2 40.0%	3 60.0%		
Hormonal	Cushing (4)	1 25.0%	3 75.0%	2.62	.27
	Non functioning (5)	1 20.0%	4 80.0%		
	Virlizing (1)	1 100.0%	0 0.0%		
Capsular	Negative (8)	3	5		.301

invasion		37.5%	62.5%	1.07	
	Positive (2)	0 0.0%	2 100.0%		
Lymphovascular invasion	Negative (5)	3 60.0%	2 40.0%	4.24	**.03(S)**
	Positive (5)	0 0.0%	5 100.0%		
Grade	High (5)	0 0.0%	5 100.0%	4.24	**.03(S)**
	Low (5)	3 60.0%	2 40.0%		
Necrosis	Focal (2)	2 100.0%	0 0.0%	5.84	**.01(S)**
	Wide (8)	1 12.5%	7 86.5%		
Atypical mitosis	Absent (5)	1 20.0%	4 80.0%	.47	**.49**
	Present (5)	2 40.0%	3 60.0%		
Diffuse architecture	Absent (2)	0 0.0%	2 100.0%	1.07	**.30**
	Present (8)	3 37.5%	5 62.5%		
clear cells less than 25%	Absent (2)	1 50.0%	1 50.0%	.49	**.47**
	Present (8)	2 25.0%	6 75.0%		
nuclear grade	High (6)	0 0.0%	6 100.0%	6.42	**.01(S)**
	Low (4)	3 75.0%	1 25.0%		
T	T2(4)	3 75.0%	1 25.0%	5.920	**.01(S)**

	T3(2)	0 0.0%	2 100.0%		
	T4 (4)	0 0.0%	4 100.0%		
N	N0 (3)	3 100.0%	0 0.0%	10.0	**.006(HS)**
	N1 (7)	0 0.0%	7 100.0%		
M	M0 (5)	3 60.0%	2 40.0%	4.148	**.03 (S)**
	M1 (5)	0 0.0%	5 100.0%		
Stage	II (2)	2 100.0%	0 0.0%	6.802	**.03(S)**
	III (3)	1 33.3%	2 66.7%		
	IV (5)	0 0.0%	5 100.0%		

FET:fisher exact test S: significant HS:highly significant

Table(4): correlation between score of Ghelin expression and study group

lesion N.=45	Score of Gherlin Expression				FET	P value
	0 N.=10 N. %	1 N.=14 N. %	2 N.=9 N. %	3 N.=12 N. %		
Adrenocortical adenoma(20)	0 0.0%	12 60%	6 30%	2 10%	61.7	.000(HS)
Carcinoma (10)	10	0	0	0		

	100.0%	0.0%	0.0%	0.0%		
Pheochromocytoma(15)	0 0.0%	2 13.4%	3 20%	10 66.6%		

FET:fisher exact test HS:highly significant

Table(5) :Relation between score of gherlin expression and pheochromocytoma

Pheo N.=15		Score of Gherline Expression			FET	P value
		Weak N.=2 N. %	Moderate N.=3 N. %	Strong N.=10 N. %		
Age	≤50 (8)	1 12.5%	0 0.0%	7 87.5%	4.29	.09
	>50 (7)	1 14.3%	3 42.9%	3 42.9%		
Sex	Female (9)	1 11.1%	3 33.3%	5 55.6%	2.38	.169
	Male(6)	1 16.7%	0 0.0%	5 83.3%		
Size groups	≤5 cm (8)	1 12.5%	2 25.0%	5 62.5%	.268	.411
	>5 cm (7)	1 14.3%	1 14.3%	5 71.4%		
weight groups	≤122gm (8)	1 12.5%	1 12.5%	6 75.0%	.670	.76
	>122 gm (7)	1 14.3%	2 28.6%	4 57.1%		
PASS	2 (7)	1 14.3%	0 0.0%	6 85.7%	15.7	**.04**
	3 (4)	0 0.0%	2 50.0%	2 50.0%		

	5 (1)	1 100.0%	0 0.0%	0 0.0%		
	7 (1)					
	10 (2)	0 0.0%	0 0.0%	2 100.0%		
	12 (1)	0 0.0%	1 100.0%	0 0.0%		
Mitosis >3/10 HPF	Negative(11)	1 9.1%	2 18.2%	8 72.7%	.852	.653
	Positive (4)	1 25.0%	1 25.0%	2 50.0%		
Atypical mitosis	Negative (12)	2 16.7%	2 16.7%	8 66.7%	.83	.65
	Positive (3)	0 0.0%	1 33.3%	2 66.7%		
Necrosis	Negative (12)	2 16.7%	2 16.7%	8 66.7%	.83	.65
	Positive (3)	0 0.0%	1 33.3%	2 66.7%		
Cellular spindling	Negative(13)	0 0.0%	3 23.1%	10 76.9%	15.0	**.001**
	Positive (2)	2 100.0%	0 0.0%	0 0.0%		
Marked nuclear pleomorphism	Negative(4)	2 50.0%	0 0.0%	2 50.0%	6.8	**.03**
	Positive (12)	0 0.0%	3 27.3%	8 72.7%		
Cellular monotony	Negative (10)	2 20.0%	2 20.0%	6 40.0%	1.06	.76
	Positive (5)	0 0.0%	1 20.0%	4 80.0%		
Large nests or diffuse growth	Negative (13)	2 15.4%	2 15.4%	9 69.2%	1.75	.48

	Positive (2)	0 0.0%	1 50.0%	1 50.0%		
High cellularity	Negative (12)	2 16.7%	2 16.7%	8 66.7%	1.03	1.0
	Positive (3)	0 0.0%	1 33.3%	2 66.7%		
Capsular invasion	Negative (12)	2 16.7%	2 16.7%	8 66.7%	1.03	1.0
	Positive (3)	0 0.0%	1 33.3%	2 66.7%		
Vascular invasion	Negative (12)	1 8.3%	2 16.7%	9 75.0%	2.62	.24
	Positive (3)	1 33.3%	1 33.3%	1 33.3%		
Hyperchromosia	Negative (10)	2 20.0%	2 20.0%	6 40.0%	1.06	.76
	Positive (5)	0 0.0%	1 20.0%	4 80.0%		
GAPP	1 (1)	0 0.0%	0 0.0%	1 100.0%	8.07	.56
	2 (2)	1 12.5%	1 12.5%	6 75.0%		
	3 (2)	0 0.0%	1 50.0%	1 50.0%		
	4 (3)	0 0.0%	1 33.3%	2 66.7%		
	6 (1)	1 100.0%	0 0.0%	0 0.0%		
T					2.65	.57
	T2(6)	0 0.0%	1 16.7%	5 83.3%		
	T3 (9)	2 22.2%	2 22.2%	5 55.6%		
Laterality					5.53	.107

	Left(9)	0 0.0%	3 33.3%	6 66.6%		
	Right(6)	2 33.3%	0 0.0%	4 66.7%		

FET:fisher exact test

Table (6): Relation of SF-1 expression and gherlin expression in adrenal lesions

Score of SF-1	Spearman correlation	P vaue
Score of Gherline Expression	-.757	<.001 (HS)

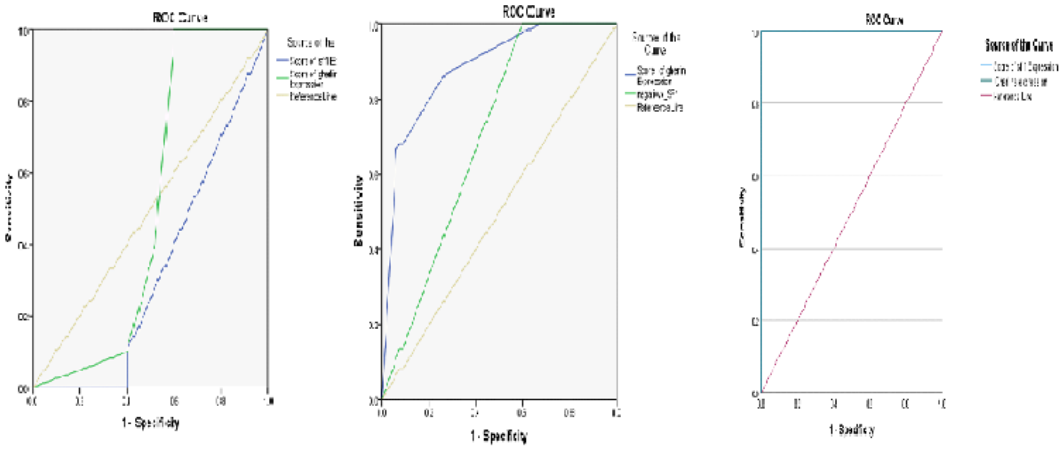


Figure (1): ROC analysis showing the use of SF-1 & gherlin immunoreactivity for differentiating adrenocortical adenoma, adrenocortical carcinoma and pheochromocytoma.

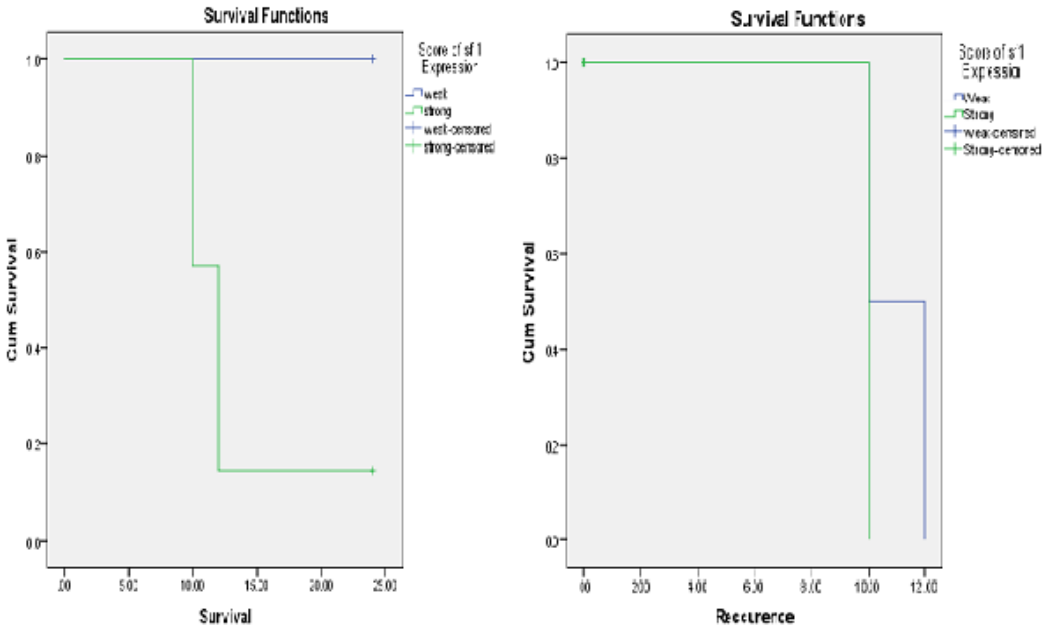
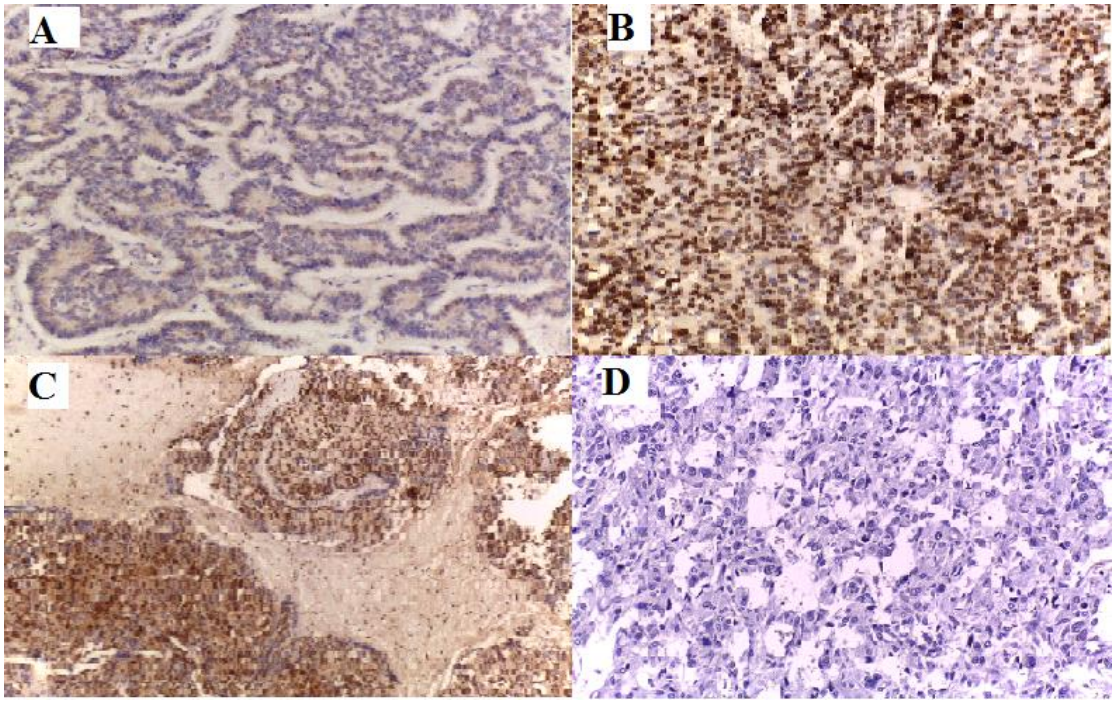


Figure (2): Relation between SF-1expression and overall survival & recurrence with A Kaplan-Meier survival analysis curve.



Figure(3): Shows SF-1 expression in adrenal lesions **(A)**: a case of adrenocortical adenoma showed negative SF-1 nuclear expression (ABC, X200). (B): a case of adrenocortical carcinoma showed diffuse strong positive SF-1 nuclear expression (ABC, X200). **(C):** a case of adrenocortical carcinoma with vascular invasion showed

diffuse strong positive SF-1 nuclear expression (ABC, X200). **(D):** a case of pheochromocytoma showed negative SF-1 nuclear expression (ABC, X200).

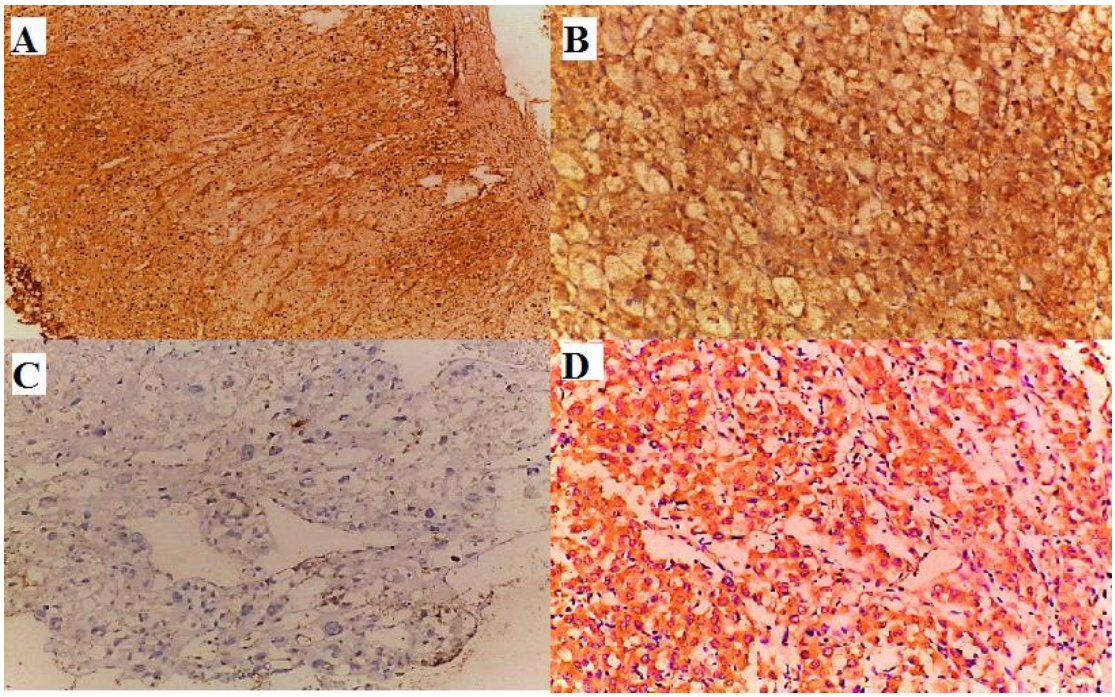


Figure (4): Shows gherlin expression in normal adrenal tissue and adrenal lesios. (A): normal adrenal cortex showed moderate positive gherlin cytoplasmic expression(ABC, X100).(B):a case of adrenocortical adenoma showed moderate positive cytoplasmic gherlin expression(ABC, X200).(C):a case of adrenocortical carcinoma showed negative gherlin expression(ABC, X200).(D): a case of pheochromocytoma strong positive cytoplasmic gherlin expression(ABCX200).