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Serum Endothelial Growth Factor a in Pancreatic Solid Lesions Diagnosed by Endoscopic Ultrasound guided Fine Needle Aspiration

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Abstract

Background: Several markers have been proposed as diagnostic markers in patients with solid pancreatic lesions, including Carbohydrate antigen 19-9 (CA 19-9), Carcinoembryonic antigen (CEA) andVascular endothelial growth factor (VEGF-A). The aim of the present study was to evaluate role of serum VEGF-A in predicting findings of Endoscopic ultrasound guided Fine Needle Aspiration (EUS-FNA) of solid pancreatic lesions and if they are correlated to each other. Patients and methods: The study included forty six patients with solid pancreatic lesion who admitted to Internal Medicine department, endoscopy unit, Faculty of Medicine, Hospitals and Kasr EL-Ainy Hospitals, Cairo University. Patients were divided into 2 groups according to FNA findings, CT abdomen and /or MRI. All patients enrolled for full history and investigated with routine laboratory studies. EUS technique was performed. Results: In our study, the mean age of group I with malignant pancreatic lesions was 56 years while the mean age of group II with benign pancreatic lesions was 44 years percentage. There was significant difference between studied groups regarding age and smoking. No significant difference was found between studied groups as regards sex, diabetes mellitus and obesity. There were statistically significant differences between the malignant group of patients classified according to EUS stage (N) regarding CA19-9 and VEGF-A however no significant difference between them regarding CEA. VEGF-A at cut off value of> 1580 u/ml has a sensitivity of 66.6% and a specificity of 94.74%in detecting advanced malignant pancreatic lesions. Conclusion: different imaging modalities are used to assess pancreatic solid lesions, among them EUS is superior to CT and MRI owing to its high ability to detect, describe and stage pancreatic solid lesions. EUS-FNA could differentiate between various pathological pancreatic neoplasms.

Keywords: EUS-FNA ; Pancreatic Solid Lesions; VEGF-A

INTRODUCTION

Pancreatic adenocarcinoma is the most common epithelial exocrinepancreatic neoplasm with a poor survival rate. Despite the advances in the research of the

molecular pathogenesis, pancreatic adenocarcinoma remains a major unsolved health problem. Overall, the 5-year survival rate is less than 5%, and only approximately 20% of the patients with resectable disease survive 5 years **(1)**. Most of the patients with pancreatic cancers are diagnosed with advanced diseases. One of the factors related to treatment failure is the high potential to develop metastasis and local recurrence. Chemotherapy is not regarded to have satisfactory results in treating pancreatic cancer, and novel approaches are required **(2)**.

Angiogenesis is crucial in the proliferation and metastasis of pancreatic cancers. Inhibitors of angiogenesis are under extensive investigation, and several prospective trials have been devoted to treat pancreatic cancer. To date, the role of the antiangiogenic therapy in pancreatic cancer is promising, but the results are not convincingly superior to the standard chemotherapeutic treatments **(3)**. Angiogenesis is essential for tumor growth and development of metastasis. The angiogenic phenotype depends on a net balance between positive and negative angiogenic factors. Vascular endothelial growth factor (VEGF) is known to be a major regulator of angiogenesis in a variety of tumors, including pancreatic cancer **(4)**.

The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). Three VEGF receptors (VEGFRs) have been identified: VEGFR-1, VEGFR-2, and VEGFR-3. Vascular endothelial growth factor is both chemotactic and mitogenic for endothelial cells and acts to increase the permeability of the vascular endothelium **(5)**. The correlation of VEGF expression and tumor progression or poor survival of pancreatic cancer has been reported. On the contrary, some of the reports in the literature did not support the prognostic role of VEGF in pancreatic cancer **(6)**.

Of note, sVEGFR-1 was present in the sera of healthy individuals, and sVEGFR1 plays a significant role in the regulation of angiogenesis by binding competitively to VEGFs as a natural VEGF inhibitor. It is thought that sVEGFR1 may function to reduce or modulate VEGF or PlGF activity in physiological and pathophysiological angiogenesis. Vascular endothelial growth factor receptor 1, including its soluble form sVEGFR1, is involved in a variety of human illnesses, making it an important target in the development of new strategies to treat disease. An inhibitory role of sVEGFR-1 in pancreatic cancer angiogenesis resulting in tumor suppression has recently been reported **(7)**.

In addition, antiangiogenic gene therapy using sFlt-1 vector can effectively inhibit angiogenesis in hepatocellular carcinoma and ovarian cancer. Although elevated serum levels of these factors have been observed in various types of human cancer, little is known regarding their roles and clinical significance in patients with pancreatic solid lesions **(8)**.

In this study, we measured the serum levels of VEGF in patients with pancreatic solid lesions and in healthy controls, then we evaluated the correlations

between these levels and clinic demographic characteristics in patients with pancreatic solid lesions.

PATIENTS AND METHODS

A Comparative cross sectional study was carried out in Internal Medicine department, endoscopy unit, Faculty of Medicine, Hospitals andKasr EL-Ainy Hospitals, Cairo University. The study included forty six patients with solid pancreatic lesion; they were divided into 2 groups according to FNA findings, CT abdomen and /or MRI. Group (I) included twenty-five patients having malignant pancreatic lesions, 16 were males (64%) and 9 were females (36%), their age ranged from 29 – 72 years with median age of 56 years. Group (II) included twenty-one patients having benign pancreatic lesion (chronic pancreatitis), 11 were males (52.4%) and 10 were females (47.6%), their age ranged from 33 – 56 years with median age of 44 years.

Inclusion criteria:

Patients with any pancreatic solid lesion diagnosed by abdominal ultrasound, abdominal CT and, or MRI in age > 18 years and of either sex.

Exclusion criteria:

Patients received blood transfusion, radiotherapy, or chemotherapy before the enrollment. Active infection or acute inflammatory disease in pancreas was excluded.

Written Informed consent was taken from the patients and their relatives to participate in the study. Approval for performing the study was obtained from Internal Medicine department, Zagazig University Hospitals after takingInstitutional Review Board (IRB) approval.

All participants were subjected to full history taking, complete physical examination, Routine lab and radiologic investigations (Chest X-ray, pelvi-abdominal ultrasound & abdominal CT and MRI) to diagnose pancreartic lesion & metastasis.

Special investigations:

- Pathological examination of EUS-FNA for suspected solid pancreatic lesions.
- Serum Amylase and lipase (normal level of amylase 30-110u/l-normal level of lipase 10-140u/l).
- Serum Carcino-embryonic antigen (CEA) (Normal range less than 5.0 ng/mL)
- CA19-9(Normal range less than 37 units/milliliter).
- Serum VEGF-A (Normal range 62 - 707 units/milliliter).

EUS-FNA technique

EUS was performed using a convex array linear echoendoscope (Pentax FG-38UX®), connected to an ultrasound equipment Hitachi-E6000®. FNA was performed with a standard 22-gauge needle (Sonotip II®, Mediglobe, Germany). Suction was released before removing the needle. The material was then spread on the slides, fixed

in 96% ethanol and processed for cytological study by Papanicolau staining. Cytology samples were evaluated for cellular preservation, background substance, cellularity, architectural integrity, and cytoplasmic and nuclear details.

Statistical Analysis:

All data were analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, Illinois, USA), MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2015). Continuous variables were expressed as the mean ± SD. The categorical variables were expressed as a number and percentage. Continuous variables were checked for normality by using Kolmogorov Smirnov test. Independent Student t (t) test. Data found to be non-normally distributed were analyzed using the Mann-Whitney U (MW) test. Percent of categorical variables were compared using the Chi-square ($\chi 2$) test. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of different markers (CEA, CA19-9, VEGF-A) for prediction of malignant pancreatic lesions.

RESULTS

The present study showed that, the mean age of group I (patients with malignant pancreatic lesions) was 56 years while the mean age of group I (patients with benign pancreatic lesions) was 44 years percentage. Statistically, there was significant difference between studied groups regarding age and smoking. On the contrast, no significant difference was found between studied groups as regards sex, diabetes mellitus and obesity **(Table 1)**. There were statistically significant differences between the malignant group of patients classified according to EUS stage (N) regarding CA19-9 and VEGF-A however no significant difference between them regarding CEA **(Table 2)**.

There were no statistically significant differences between the malignant group of patients classified according to TNM staging regarding any of laboratory data **(Table 3)**. VEGF-A at cut off value of> 1580 u/ml has a sensitivity of 66.6% and a specificity of 94.74%in detecting advanced malignant pancreatic lesions **(Table 4)**.

Table (1): Comparison of risk factors between studied groups (n=46).

	Group (I) Patients with Malignant pancreatic lesions (n=25)	**Group (II) Patients with Benign pancreatic lesions(n= 21)**	**Test**	***P***
Age (Years) ***Median(Range)***	**56 (29 – 72)**	**44 (33 – 56)**	**MW90**	**0.0001(HS)**
Sex ***Male***	**16 (59.3%)**	**11(40.7%)**	χ^2	**0.430 (NS)**

Female	9 (47.4%)	10(52.6%)	0.622	
Smoking *No* *Yes*	8 (32%) 17 (68%)	14 (66.7%) 7 (33.3%)	χ^2 0.622	0.020 (S)
Obesity *No* *Yes*	7 (28%) 18 (72%)	9 (38.1%) 12 (61.9%)	χ^2 0.518	0.47 (NS)
Diabetes mellitus *No* *Yes*	13 (52%) 12 (48%)	9 (42.9%) 12 (57.1%)	χ^2 0.374	0.54 (NS)

MW = Mann-Whitney U test , χ^2 Chi-squared test, A *p* value <0.05 was considered statistically significant(S).

Table (2): Comparison of different tumor markers in malignant group of patients classified according to EUS stage (N) (n=25)

	N0 (n= 10)	N1 (n=9)	N2 (n= 6)	Test	*P*
CEA (ng/mL) *Median (Range)*	7.75 (2.9 – 17)	7 (1.2 – 40)	8.1 (1.7 – 23)	KW 2.62	0.45 (NS)
CA19-9 (u/mL) *Median (Range)*	106 (13– 4011)	60 (5.2– 900)	128.5 (15 – 1502)	KW 12.3	0.006 (S)
VEGF-A(u/mL) *Median (Range)*	834 (477 – 1289.2)	1233 (142 – 2028.2)	1924 (920.7 – 3791.3)	KW 25.5	0.00001 (HS)

KW = Kruskal-Wallis test.

Table (3): Comparison of different basic laboratory data in malignant group of patients classified according to (TNM) staging system (n=25)

	Stage I (n= 9)	Stage II (n=10)	Stage III (n= 6)	Test	*P*
WBCs($x10^3/mm^3$) *Median (Range)*	4.9 (3.8 – 11)	7.25 (4– 11)	5.8 (4 – 10)	KW 2.58	0.27 (NS)
Hemoglobin (g/dL) *Median (Range)*	11.5 (11– 13)	11(10.3– 13.5)	12.1 (11 – 14)	KW 4.2	0.104 (NS)

Platelet count ($x10^3/mm^3$) *Median (Range)*	**211 (151 – 331)**	**204 (129 – 461)**	**196 (165 – 411)**	**KW 0.07**	**0.9 (NS)**
INR *Mean± SD*	**1.05 ± 0.072**	**1.05 ± 0.052**	**1.08 ± 0.117**	**F 0.36**	**0.701 (NS)**
Creatinine (mg/dL) *Mean± SD*	**0.73 ± 0.15**	**0.70 ± 0.066**	**0.78 ± 0.24**	**F 0.5**	**0.589 (NS)**
Total Bilirubin (mg/dL) *Median (Range)*	**2.8 (0.7 – 3.9)**	**4.15 (0.7 – 20)**	**0.8 (0.4 – 5.2)**	**KW 5.89**	**0.05 (NS)**
Direct Bilirubin (mg/dL) *Median (Range)*	**1.9 (0.5– 2.7)**	**3 (0.4 – 18)**	**0.4 (0.2– 4.5)**	**KW 5.47**	**0.06 (NS)**
Amylase (mg/dL) *Median (Range)*	**13 (5.2 – 307)**	**14.7 (7 – 982)**	**32.5 (3.5 – 200)**	**KW 1.46**	**0.48 (NS)**
lipase (mg/dL) *Median (Range)*	**22 (6.5 – 137)**	**31.5 (6.5 – 96)**	**20.25 (13 – 55)**	**KW 0.2**	**0.86 (NS)**

KW = Kruskal1-Wallis test, F= One way ANOVA

Table (4): Specificity and sensitivity of VEGF-A in predicting advanced malignancy stage III among malignant group of patients (n= 25).

Cut-off values	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC (95% CI)	Accuracy %
VEGF-A >1580 u/ml	**66.6% (22.3 – 95.7)**	**94.74% (74 – 99.9)**	**80 % (35.4 – 96.7)**	**90% (74.3 – 96.6)**	**0.86** (0.663– 0.97)**	**79.45 %**

***p<0.2601 (NS)** ****p=0.001(S)** *** **p=0.0013 (S)**

DISCUSSION:

The present study was conducted on 46 patients with malignant and benign pancreatic lesions who admitted to endoscopy unit, Faculty of Medicine, Hospitals and Kasr EL-Ainy Hospitals, Cairo University for performance of Endoscopic Ultrasound Technique. This comparative clinical trials was aimed to evaluate evaluate role of serum VEGF-A in predicting findings of EUS-FNA of pancreatic tumors and if they are correlated to each other.

In our study, there was a significant difference regarding age and smoking between the studied groups. This constant with **Raimondi et al. (9)** who stated that pancreatic cancer is strongly age-dependent however the median age of pancreatic cancer diagnosis was 72 years and only about 5–10% of patients develop pancreatic cancer before the age of 50 years, but this group is possible to involve patients with underlying predisposing genetic disorders. The younger age in our Egyptian patients may be due to different demographic and intrinsic biologic factors. the fact that smoking is the most common known risk factor and is the cause of 20–25% of all pancreatic tumors. Also, our results are agree with **Wahi et al., (10)** found the cumulative likelihood of developing pancreatic cancer is about 1% for men and slightly less for women since smoking rates are higher in men than in women, smoking could be responsible for the higher incidence observed in men.

Regarding obesity, 72% of patients with malignant pancreatic lesions were obese, and only 28% were non obese.But there wasn't a significant difference between studied groups. **Larsson et al. (11)** stated thatincreased BMI wasassociated with an increased risk of pancreatic cancer; this may be attributed to the small sample size in our study. The mechanism may be direct tumor promotion or anindirect effect via a link between obesity and inflammatory responses **(12).**

In our study, EUS findings were highly significant in differentiating benign from malignant lesions. This finding copes with the fact that EUS with or without fine-needle aspiration has become the principal technique for assessing pancreaticobiliary disorders and has shown to have a higher diagnostic yield than CT, and transabdominal ultrasound for recognizing early pancreatic tumors. As a diagnostic modality for pancreatic cancer, EUS has proved rates higher than 90%, especially for lesions less than 2-3 cm in size in which it reaches a sensitivity rate of 99% versus 55% for CT **(13)**.

In our study, there was a significant difference between the studied populations grouped according to pathological nature regarding CT findings. Moreover, there was a significant difference between the studied populations in malignant group according to EUS stage (T), (N) regarding CT findings. **Hunt et al. (14)** stated that EUS is considered an indispensable tool for the detection, characterization, and differential diagnosis of solid pancreatic lesions, including pancreatic ductal adenocarcinoma. **Agarwal et al. (15)** documented that compared with CT, EUS can detect about 14% of pancreatic cancers that were missed on CT. Furthermore, a meta-analysis evaluated the performance of EUS in those patients without an obvious mass on CT but with clinical suspicion for pancreatic malignancy, and showed a higher sensitivity of EUS for detecting a pancreatic neoplasm **(16)**.

As regard VEGF -A, serum level of VEGF-A increases significantly from localized lesion to infiltrating and metastasizing lesions. our results could be elucidated in the light of **Liang et al. (17)** study who found out that the expression of

VEGF on mRNA and protein levels in pancreatic cancer tissues was obviously higher than in adjacent normal pancreatic tissues, which suggested that the expression of VEGF plays an important role in the occurrence of pancreatic cancer and the expression intensity of VEGF was related to tumor size, TNM stage, and lymph node metastasis. This finding made VEGF -A more valuable as a diagnostic and prognostic tool than CA 19-9. Also, **Tang et al. (18)** study found the same findings where Immunohistochemical analysis of 50 pancreatic cancer tissue samples revealed the presence of VEGF-Aimmune reactivity in 50% of the cancer tissue samples. The presence of VEGF-A in these cells was associated with larger tumor size and enhanced local spread but it was not associated with decreased patient survival.

In a study of **Meng et al. (19)** indicated that elevated serum CEA level, as a vital supplementary to CA19-9, can play an important role in the clinical diagnosis of pancreatic cancer patients and predict poor prognosis.

In contrast to our findings, **Kato et al. (20)** stated that CEA is a crucial prognostic indicator for localized pancreatic ductal adenocarcinoma. This disagreement with our results could be explained by the fact that CEA elevated in a wide range of benign as well malignant conditions e.g. chronic obstructive pulmonary disease, diabetic nephropathy, type 2 DM, age over 65 years, asthma, cerebrovascular disease, gastric ulcer, gout, hyperuricemia and pancreatitis **(21)**.

Therefore, The fundamental role of VEGF-A in predicting malignancy among pancreatic solid lesions confirmed in our study through the univariable and multivariable logistic regression analysis, where post-adjustment of the different variables.

CONCLUSION:

We can conclude that different imaging modalities are used to assess pancreatic solid lesions, among them EUS is superior to CT and MRI owing to its high ability to detect, describe and stage pancreatic solid lesions. EUS-FNA could differentiate between various pathological pancreatic neoplasms. VEGF-A was only the predictor for malignancy.

No Conflict of interest.

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