The Application of ImmunohistoChemical Markers for Diagnosis, Classification and Grading of Neuroendocrine Tumors

تطبيق المعلّمات المناعية الكيميائية النسيجية لتشخيص وتصنيف وتحديد درجة أورام الغدد الصم العصبية

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الخلاصة:

الهدف : لدراسة حساسية ال(Chromogranin A) في تشخيص اورام الغدد الصم العصبية من مواقع مختلفة في الجسم وارتباطه مع المعايير المرضية والسريرية مثل موقع الورم والصف.

المنهجية : طبقت هذه الدراسة على خمسين مريض مصاب بأورام الغدد الصم العصبية في قسم علم الأمراض، كلية الطب ، جامعة الكوفة من كانون الثاني 2013 الى تشرين الأول 2013.. أعمارهم تتراوح ما بين 22-80 سنة وكان المعدل العمري 48.3. استخدمت طريقة Two step Envision ^{TM} flex لتحديد التعبير المناعي لمستضد ki67 (MIB-1) و Chromogranin A.

النتائج : كان الموقع الرئيسي للمرضى الذين يعانون من أورام الغدد الصم العصبية المنخرطين في هذه الدراسة هو الجهاز الهضمي المعوي والبنكرياس وكانت هناك هيمنة الإناث، كان التعبير المناعي لل Chromogranin A هو 44 (88%) ولاتوجد علاقة ذات مغزى بين التعبير المناعي لل Chromogranin A مع الموقع والصف للأورام.

الاستنتاجات : Chromogranin A معلم حساس للغاية في تشخيص أورام الغدد الصم العصبية بصرف النظر عن الموقع والصف.

التوصيات : ننصح باستخدام المعلم المناعي Chromogranin A كمعلم رئيسي في تشخيص الأورام الغدد الصم العصبية بالإضافة الى إمكانية استخدامه كهدف لنمط جديد في علاج الأورام .

Abstract :

objectives: To study the sensitivity of Chromogranin A in the diagnosis of neuroendocrine tumors from various locations in the body and its association with clinicopathological parameters like site and grade of tumors .

methodology: This study is conducted in the Department of Pathology, Faculty of Medicine, University of Kufa from January - October 2013.

A total of 50 patients with neuroendocrine tumors from different locations. their ages ranging between 22-80 years with a mean age of 48.3 years. Two step Envision ^{TM} flex method was employed for immunohistochemical detection of ki67 antigen (MIB -1) and Chromogranin A .

Results: Main site for patients with neuroendocrine tumors included in this study is gastroenteropancreatic system and there is female predominance , from 50 patients 44 (88%) cases was positive for Chromoranin A expression , there was no significant differences in expression of the Chromogranin A with location and grade of tumors (P value >0.05)

Conclusion: Chromogranin A is highly sensitive marker in diagnosis of neuroendocrine tumors irrespective to tumors location and tumor grade .

Recommendation: We advise to use Chromogranin A immunohistochemical marker as a main marker in diagnosis of neuroendocrie tumors in addition to that this marker can be used as target for new modality in tumor therapy .

Keywords: neuroendocrine tumor ,chromogranin A, ki 67

INTRODUCTION :

Neuroendocrine tumors (NETs) are relatively rare that arises from the cell of the diffuse neuroendocrine system that scattered in all organs mainly gastrointestinal tract , bronchopulmonary system and others . The annual incidence is approximately 2.5-5 per 100,000. The prevalence has been estimated as 35 per 100,000, and may be considerably higher if clinically silent tumors are included**.** (1)

Most NETs have the ability to produce and secrete a variety of peptide hormones and amines which cause clinical syndromes and are used as markers for the diagnosis of NETs . The diagnosis of neuroendocrine tumor patients with absence of any symptoms depend on detection general NET markers, including the secretory granule proteins (chromogranins A), the synaptic vesicle membrane glycoprotein synaptophysin) and the cytosolic marker (neuron specific-enolase)**.**(2)

Without correct diagnosis the tumor can be falsely diagnosed as adenocarcinoma, with a negative impact patient regarding management and underestimation of prospects for survival.(3) there are many classification system for neuroendocrine tumors but the newest one was in 2010 which separates neuroendocrine tumors into two main groups: well-differentiated NET (grade 1, grade 2) and poorly differentiated NET (G3) neuroendocrine carcinoma (NEC), including large cell and small cell neuroendocrine carcinoma (LCNEC and SCNEC) that quietly differ in their biological behavior and lines of therapy**.** (4)

OBJECTIVES :

To study the sensitivity of Chromogranin A in the diagnosis of neuroendocrine tumors from various locations in the body and its association with clinicopathological parameters like site and grade of tumors .

METHODOLOGY :

This study is conducted in the Department of Pathology, Faculty of Medicine, University of Kufa from January - October 2013.A total of fifty formalin-fixed paraffin-embedded tissues sections of various organs from histopathologicaly confirm neuroendocrine tumors Iraqi patients were included Thirty five cases from gasteroenteropancreatic system, 9 cases from bronchopulmonary system , 6 cases from other sites, were retrieved from the lab unite of al-Sader medical city in Al Najaf and some of private labs in al-Najaf and Baghdad provinces**.**

From each tissue block, 3 sections of 4 µm thickness were taken. One section was stained with hematoxylin and eosin ,for revision of the histopathological diagnosis, and

the other 2 sections were stained immunohistochemically using two step Envision ™ flex method for Ki-67 Antigen (monoclonal mouse antihuman ki67 antigen, clone MIB-1 , Dako Denmark , was used at dilution 1:75.it is nuclear marker) and Chromogranin A (monoclonal mouse antihuman Chromogranin , Dako Denmark, used at dilution of 1:400, it is cytoplasmic markers).

With each run of immunostaining positive and negative control sections were processed, Positive control pancreatic tissues sections which are known to express chromogranin A and small intestine for ki67, while Negative controls were obtained by omitting the primary antibody for the used markers.

The criterion for positive immunoreaction is dark brown precipitate (cytoplasmic for Chromogranin A , and nuclear for Ki 67). While the intensity of chromogranin A staining was assessed by counting the percentage of positive cells in 100 malignant cells at objective 40 total magnification. The immunostaining was calculated as the percentage of immunoreactive cells per total number of malignant cells. Each sample was scanned for at least five fields with

a high power magnification, chromogranin A assessed as positive or negative (**Positive staining for chromogranin A should be considerd if >10% of tumor cells was positive).**[5]

Table (1) : ki7 scoring system:

Grade	Staining pattern
Grade I	≤2% of tumor cells are positive
Grade II	3%–20% of tumor cells are positive
Grade III	> 20% of tumor cells are positive

Table (1) show ki67 scoring system was assessed as percentage of immunostained cell in a total 2,000 tumor cells in areas of highest nuclear labeling (hot spot)at magnification 40 x. A low Ki-67 index was defined as ≤2% positive cells, an intermediate index as(3–20%)positive cells and a high proliferation index as > 20% positive cells according to recent guidelines from World Health Organization (WHO) and the European Neuroendocrine Tumor Society (ENET) recommendations.[6]

RESULTS :

The age of patients included in this study ranged from 22-80 years with a mean age of(48.3±13.8) years (mean± SD) and peak age group being 41-50 years (26%). (Figure 1). Consisting of females 28 (56%) outnumber the males 22 (44%).(Figure 2)

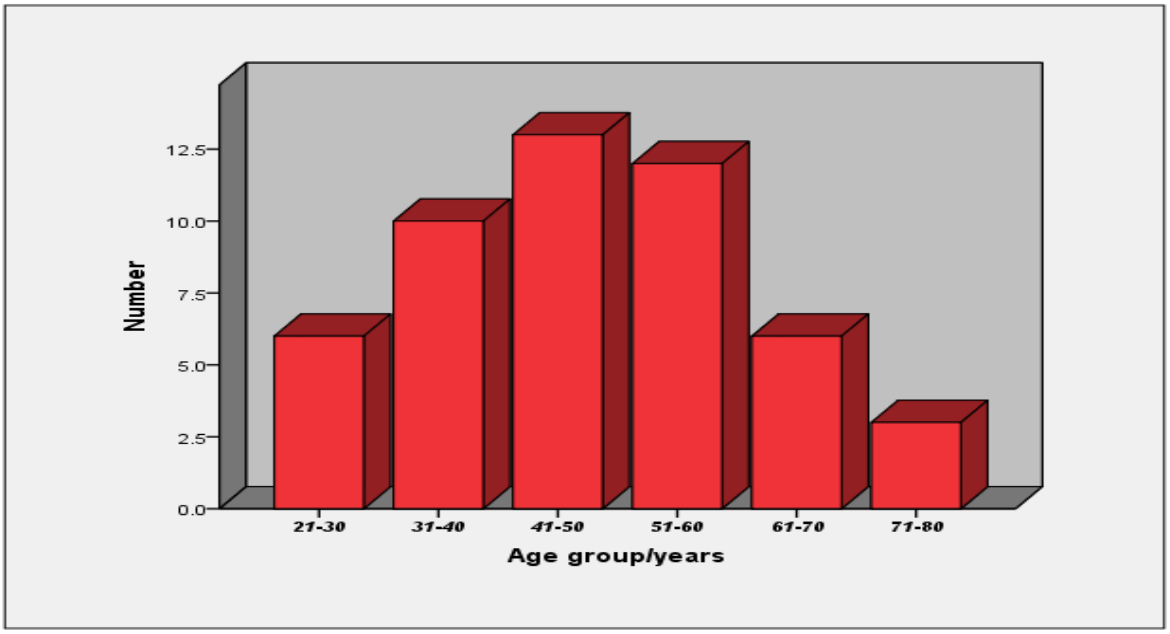
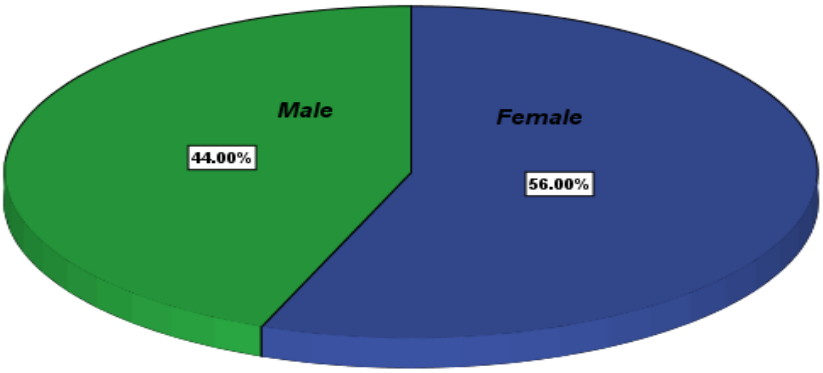


Figure (1): Age distribution of the presented neuroendocrine tumor patients



Figure(2): Gender distribution among presented neuroendocrine tumor patients.

Table (2) :Location of tumor in the presented neuroendocrine tumors patients

Location of tumors	No. patient	Percentage
Gasteroenteropancreatic system	35	70%
Bronchopulmonary system	9	18%
others	6	12%
Total	50	100%

Table (2) show Site distribution revealed that (35) (70%) cases are taken from Gastroenteropancreatic (GEP) system, (9) (18%) cases from Bronchopulmonary system, (6) (12%) cases from other sites.

Table(3) : Distribution of tumors within Gastroenteropancreatic system

Site	No. patient	Percentage
Stomach	12	34%
Pancreas	11	31%
Small intestine	9	26%
Colon	1	3%
Rectum	2	6%
Total	35	100%

Table (3) explain the assessement of site distribution within Gastroeneropancreatic neuroendocrine tumors ,the result revealed that (12 out of 35)(34%) cases are from stomach, (11 out of 35)(31%) from pancreas, (9 out of 35)(26%) from small intestine, (1 out of 35) (3%) from colon and (2 out of 35)(6%) from rectum.

Table (4): Distribution of GEP –NET patients according to the grade.

Grade of tumors	No. of patients	Percentage
Grade I (Ki 67≤2)	**9**	**26%**
Grade II (Ki 67 3-20)	**15**	**43%**
Grade III (Ki67 >20)	**11**	**31%**
Total	**35**	**100%**

Table (4) show Grading of Gasteroenteropancreatic neuroendocrine tumors (GEP-NET) were assessed according to the WHO grading system 2010, revealing that grade I was reported in (9)(26%) of cases, grade II in (15) (43%), while those of grade III were (11) (31 %) cases.

Table (5): Immunohistochemical expression of Chromogranin A in presented neuroendocrine tumor patients.(negative result include both score 0 and +1)

Type of tissue	Immunostaining of chromogranin A		Total	P value
	Positive	Negative		
GEP –NET	32(91.43%)	3(8.57%)	35(70%)	0.820
Bronchopulmonary system	7(77.78%)	2(22.22%)	9(18%)	
Others	5(83.33)%	1(16.67%)	6(12%)	
Total	44(88%)	6(12%)	50(100%)	

Table (5) show that Chromogranin A expression reported in (44 out of 50 cases) (88%) and only (6 out of 50 cases)(12%) was negative for Chromogranin A. assessment of Chromogranin A expression in neuroendocrine tumors from different location revealed that: Gastroenteropancreatic neuroendocrine tumors (32 out of 35cases)(91%)was positive for Chromogranin A, only (3 out of 35 cases)(8,57%)was negative, Bronchopulmonary neuroendocrine tumors (7 out of 9 cases) (77%78%) was positive and (2 out of 9 cases)(22,22%) was negative , in other sites only (1 out of 6 cases) (16.67%) was negative while the remaining 5 cases was positive with (83.33%). There was no significant differences in expression of chromogranin A among different neuroendocrine tumors in various locations.($p>0.05$) (Figures 3,4)

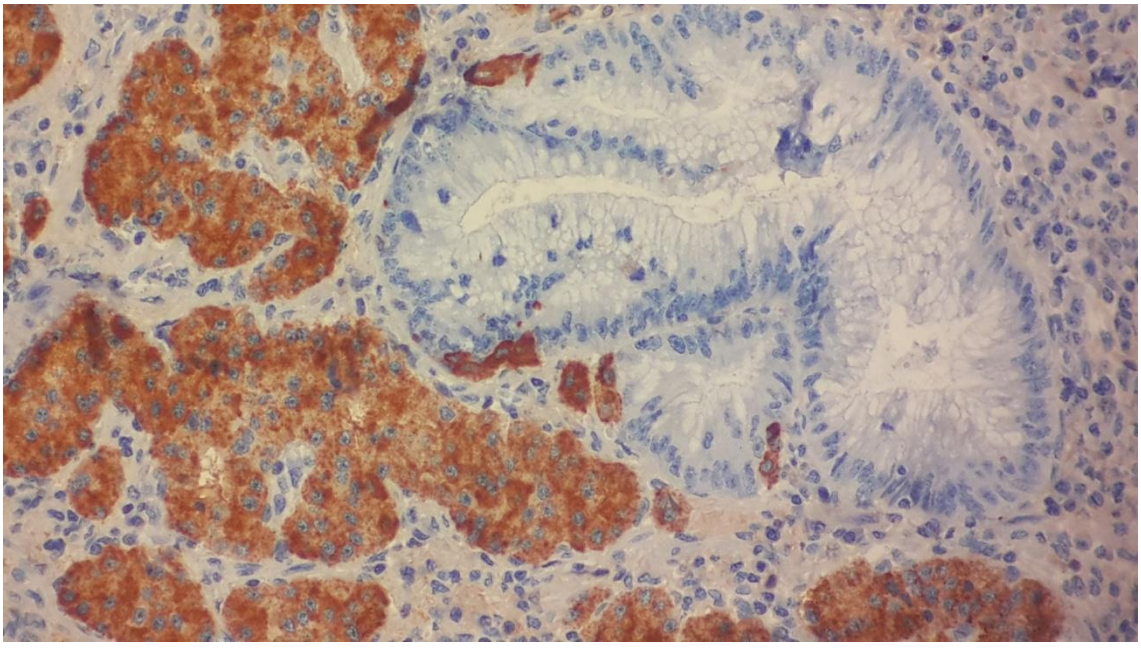


Figure (3): Neuroendocrine tumor, showing strong cytoplasmic staining of chromogranin A . with presence of intact mucin secreting glandular structure in case of intestinal neuroendocrine tumor .[40X]

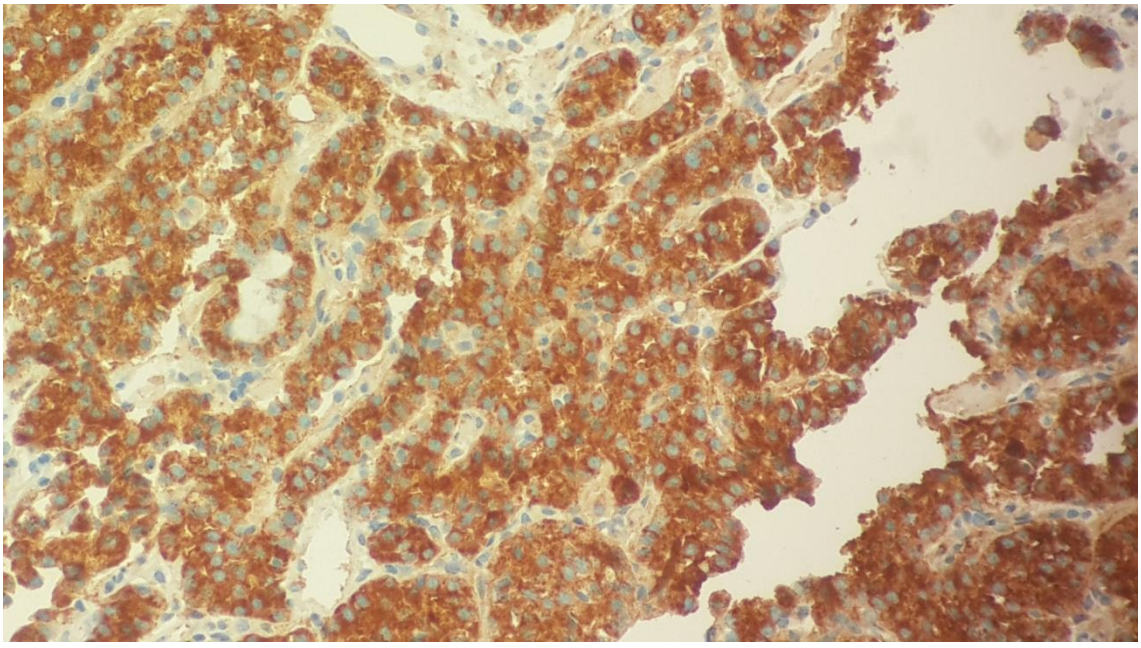


Figure (4): Neuroendocrine tumor with trabecular pattern of growth with strong staining for chromogranin A . and negative staining of nucleus [40X]

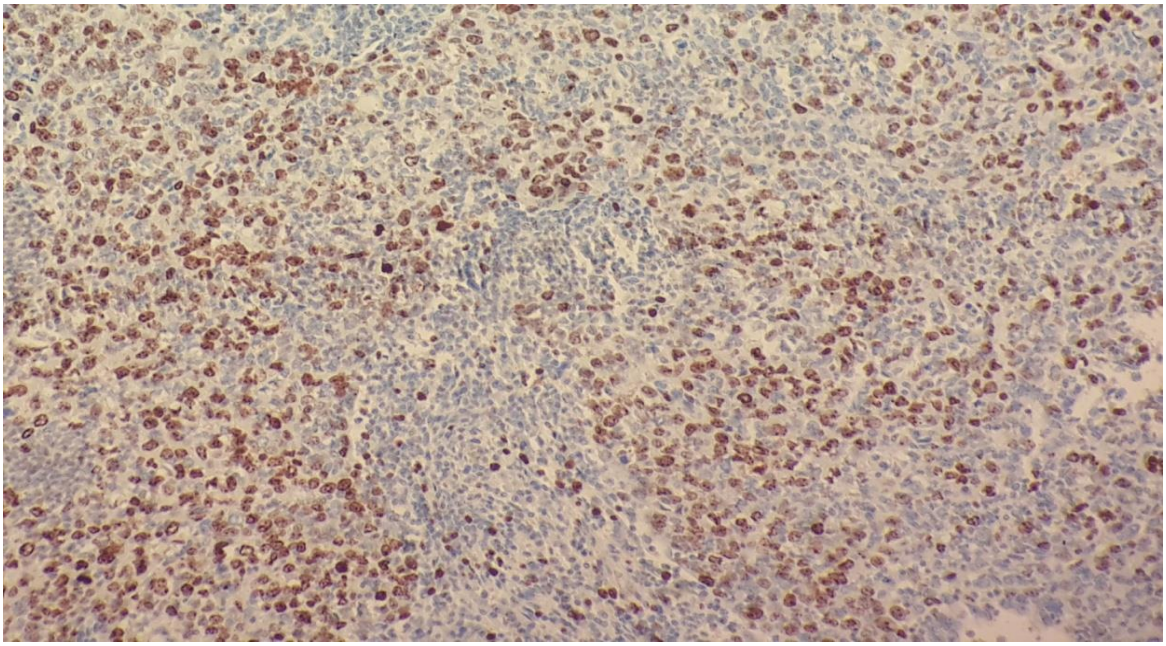


Figure 5: Neuroendocrine tumors, showing grade III neuroendocrine tumors with >20%of tumor cells positive nuclear stain for ki67 [10X]

Table(6) : Immunostaining of Chromogranin A in relation to grade of tumor.(negative result include both score 0 and +1)

Grade	Immunostaining Chromogranin A		Total	P value
	Positive	**Negative**		
Grade I	8(88.89%)	1(11.11%)	9(25.71%)	**0.609**
Grade II	15(100%)	0	15(42.86%)	
Grade III	9(81.82%)	2(18.18%)	11(31.43%)	
Total	**32(91.43%)**	**3(8.57%)**	**35(100%)**	

Table (6) demonastrae that Immunohistochemical analysis of the Chromogranin A protein expression in relation to grade of tumor revealed that positive result of Chromogranin A was reported in (8 out of 9 cases) of grade I ,all of cases in grade II was positive for Chromogranin A (15 out of 15) , (9 out of 11) of grade III . There was no significant differences between Chromogranin A expression and grade of tumors.($p>0.05$)

DISCUSSION:

Neuroendocrine tumors relatively rare tumors but with continues increase in the incidence that seem to be exceed that of breast and lung , especially this changes occurred on basis of tumors location with increase in primary tumors of stomach ,rectum and small intestine . [7] This point bring the attention to study this tumors from different point of view. Immunohistochemical detection of general neuroendocrine marker (chromogranin A, synaptophysin, neuron specific enolase) is considered as most important diagnostic tool for neuroendocrine tumors ,so in this study, we investigated the immunohistochemical detection rate of chromogranin A (large secretary granule associated marker)to determine its diagnostic sensitivity and its relation to grade and location of tumors. Clinicopathological assessment

revealed that gender distribution show that male cases were 24 (48%) & female cases were 26 (52%) and male to female ratio in the current study was (0.78:1) , this matching with other study .[1] and close to .[8] with (58%) female and (42%) male. In the current study patients age ranges from (22-80) years with a mean of 48.38, which is seem to be similar to other studies. [9] [10]

Site distribution (70%) of cases were taking from Gastroenteropancreatic (GEP) system, (18%) were taking from Bronchopulmonary system & (12%) from other sites, this pattern of site distributiongoing with previous studies.[7] [11]

Within gastroenteropancreatic neuroendocrine tumors the stomach was the main site with (34%) this look to be similar with previous studies.[12] [8]

Regarding grade distribution within Gastroenteropancreatic system Most of cases in the current study located within Grade II group (15 out of 35) (43%) in reverse with other studies most of cases located within Grade I group .[13] [14] This variation in grade distribution within GEP-NET may be due to different grading system that used in the studies. Immunohistochemical expression of Chromogranin A have clarified that (44 out of 50 cases) (88%) of neuroendocrine tumors were expressing Chromogranin A in their histological sections These results have been reported by many investigators over the world. In agreement with many other studies reported .[15] [8].

In neuroendocrine tumors from different locations , it has been that chromogranin A was positive in (91.4%) of gastroenteropancreatic NET, 77,8% of bronchopulmonary NET and 83.3% of others sites ,without significant difference in chromogranin A expression between different sites (p value >0.05) these result are corresponding to other study.[16] that concern with immunohistochemical detection of chromogranin A specifically in neuroendocrine carcinoma (grade III) .

The immunohistochemical analysis of the Chromogranin A in relation to grade show that immunoexpressionwas noticed in (88.9%) of grade I Gastroenteropancreatic neuroendocrine tumors , and in (100%) of grade II group Of Gastro entero pancreatic neuroendocrine tumors . In grade III, 81.8% of them revealed positive within grade of tumor . Despite there is higher percent with lower grade but There was no significant difference among these grades (P value <0.05). This is also supported by literature.[8] [17]

CONCLUSION:

From above result we conclude that chromogranin A is highly sensitive marker in diagnosis of neuroendocrine tumors irrespective to tumor grade and location.

RECOMMENDATION:

We advise to use Chromogranin A immunohistochemical marker as a main marker in diagnosis of neuroendocrine tumors in addition to that his marker can be used as target for new modality in tumor therapy .

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