

Prognostic value of somatostatin receptor-2 positivity in gastroenteropancreatic neuroendocrine tumors in reference to known prognostic factors

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Background/aims: Identification of the predictive factors for the prognosis of gastroenteropancreatic neuroendocrine tumors is important but rather challenging due to the rarity of the condition. This study aimed to examine the association between somatostatin receptor-2 positivity and known prognostic factors for gastroenteropancreatic neuroendocrine tumor to identify the value of somatostatin receptor-2 positivity itself as a predictive factor for prognosis. **Materials and Methods:** Records of 41 gastroenteropancreatic neuroendocrine tumor patients (24 females, 17 males) were retrospectively reviewed. The relations between somatostatin receptor-2 positivity and known prognostic factors including tumor stage, Ki-67 positivity, vascular or perineural invasion, lymph node metastasis, presence of necrosis, and soft tissue extension were analyzed. **Results:** Sixty percent of the patients had histologically confirmed somatostatin receptor-2 positivity with 45% exhibiting focal and 15% showing diffuse staining characteristic. No significant relation was found between somatostatin receptor-2 positivity and any of the known prognostic factors for gastroenteropancreatic neuroendocrine tumor: versus stage, $p=0.67$; vs. lymph node metastasis, $p=0.51$; vs. vascular invasion, $p=0.11$; vs. extension to surrounding soft tissue, $p=0.54$; vs. necrosis, $p=0.23$; vs. lymphatic invasion, $p=0.25$; and vs. perineural invasion, $p=0.42$. **Conclusions:** Somatostatin receptor-2 positivity, either focal or diffuse, does not seem to predict prognosis in gastroenteropancreatic neuroendocrine tumors. However, growing evidence supports the benefits of somatostatin analogues as adjunctive treatment in this group of patients.

Key words: Somatostatin receptor-2, gastroenteropancreatic neuroendocrine tumors, prognosis, somatostatin analogues

Gastroenteropankreatik nöroendokrin tümörlerde prognostik faktörler ile somatostatin reseptör 2'nin ilişkisi

Giriş ve Amaç: Gastroenteropankreatik nöroendokrin tümörlerde prognozu belirleyen prediktif faktörlerin saptanması önemli olmakla birlikte nadir bir hastalık olması sebebiyle zordur. Bu çalışmanın amacı; somatostatin reseptör-2 pozitifliği ile gastroenteropankreatik nöroendokrin tümörün tanımlanmış prognostik faktörleri ile olan ilişkisini araştırarak somatostatin reseptör-2 pozitifliğinin prognostik önemini ortaya koymaktır. **Gereç ve Yöntem:** Gastroenteropankreatik nöroendokrin tümör tanısı olan 41 hastanın (24 kadın, 17 erkek) verileri retrospektif olarak incelendi. Bilinen prognostik faktörlerden olan Ki-67 pozitifliği, vasküler veya perinöral invazyon, lenf nodu metastazi, nekroz varlığı ve yumuşak doku yayılımı ile somatostatin reseptör-2 pozitifliği arasındaki ilişki analiz edildi. **Bulgular:** Hastaların %60'ında somatostatin reseptör-2 pozitifliği saptandı. Bunların %45'inde focal, %15'de difüz pozitiflik mevcuttu. Prognostik faktörler ile somatostatin reseptör-2 pozitifliği arasında anlamlı bir ilişki saptanmadı. İstatistiksel analizde saptanan sonuçlar şunlardı; evre $p=0.67$, lenf nodu metastazi $p=0.51$, vasküler invazyon $p=0.11$, çevre yumuşak doku yayılımı $p=0.54$, nekroz $p=0.23$, lenfatik invazyon $p=0.25$ ve perinöral invazyon $p=0.42$. **Sonuç:** Fokal ya da difüz somatostatin reseptör-2 pozitifliği, gastroenteropankreatik nöroendokrin tümörde prognoza etkili bir faktör olarak bulunmamıştır. Ancak giderek artan sayıdaki çalışmalar, bu grup hastaların tedavisinde somatostatin analoglarının etkili olduğunu desteklemektedir.

Anahtar kelimeler: Somatostatin reseptör-2, gastroenteropankreatik nöroendokrin tümörler, prognoz, somatostatin analogları

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INTRODUCTION

Neuroendocrine tumors (NETs) are slow-growing tumors secreting peptides, mainly serotonin. They can develop in any part of the body and are able to metastasize (1). Gastroenteropancreatic neuroendocrine tumors (GEP NETs), on the other hand, are rare tumors, with an incidence about 4-5/1,000,000, which originate from the diffuse neuroendocrine system of the gut. The most common GEP NETs are insulinomas, gastrinomas, and tumors causing carcinoid syndrome. Incidence of GEP NETs has increased more than two-fold during the last 16 years, whereas the incidence of pancreatic adenocarcinoma was constant during this period (2).

Somatostatin is a peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation. Its 28 amino acid form, intestinal somatostatin, which is secreted from the gastrointestinal canal and pancreas, inhibits or antagonizes many gastrointestinal hormones. Somatostatin exhibits its effects through binding somatostatin receptors (SSTR) in the target tissue. There are five known somatostatin receptors: SSSTR-1, SSSTR-2, SSSTR-3, SSSTR-4, and SSSTR-5. Although the half-life of natural somatostatin is very short (<3 minutes), precluding its use in the treatment of hormone-secreting endocrine tumors, the more stable somatostatin analogues octreotide and lanreotide, with longer half-lives, are used for the management of these conditions.

Most GEP NETs express SSSTRs, with SSSTR-2 being the most dominant type (3,4). Identification of these receptors in GEP NETs represents an advance in their management since somatostatin analogues have become the most effective palliative agents (5). Long-acting synthetic somatostatin analogues such as octreotide and octreotide-LAR (long-acting release) can provide symptomatic relief through inhibiting the hormones responsible for the clinical symptoms by binding to the SSSTRs on the tumor cell membrane (6).

Since GEP NETs are very rare tumors, identification of the predictive factors for prognosis is rather challenging. Given the tumors expressing SSSTR-2 receptor are known to respond to the treatment with somatostatin analogues, the high level of SSSTR-2 expression in GEP NETs may well have implications on prognosis through modifying the biological behavior of the tumor and/or clinical course of the disease. If so, then testing SSSTR-2 re-

ceptor positivity may guide in the management of the disease.

This study aimed to examine the association between SSSTR-2 positivity and known prognostic factors for GEP NET, including stage, Ki-67 positivity, vascular or perineural invasion, lymph node metastasis, presence of necrosis, and soft tissue extension, in an attempt to identify the value of SSSTR-2 positivity itself as a predictive factor for prognosis.

MATERIALS AND METHODS

Patients

The medical records of 41 GEP NET patients (24 females, 17 males) operated in the General Surgery Clinic of Ege University Medical Faculty between 1996 and 2008 were retrospectively reviewed. Patients had undergone pancreaticoduodenectomy, distal pancreatectomy, or enucleation.

Diagnosis

After establishing a clinical diagnosis, all patients underwent Doppler ultrasonography (USG) prior to surgery. Computerized tomography (CT) was the second most preferred diagnostic tool and was used in 23 patients (56.1%). Magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) were used to visualize the biliary tree or a suspicious soft tissue, or to differentiate a liver mass from hemangioma, when necessary (n=4, 9.8%). Endoscopic retrograde cholangiography was done in three patients (7.3%) and somatostatin receptor scintigraphy (SRS) was done in 18 patients (43.9%). Although a predefined algorithm was preferred in our center, the order of diagnostic investigations showed variation in patients referred from other institutions.

Staining and histologic evaluation was carried out by obtaining new sections from the paraffin blocks in the Pathology Department. All the sections were evaluated by means of World Health Organization (WHO) tumor grading, perineural, vascular and lymphatic invasion, lymph node metastasis, tumor necrosis, tumor radius, and mitosis count under 10x magnification by regular light microscopes. Ki-67 (MIB-1, Dako, Denmark) and SSSTR-2 (AB9486, Chemicon, USA) were studied by avidin-biotin complex method (KP-50 A Chemicon, USA). SSSTR-2 staining pattern (membranous, cytoplasmic) and prevalence were evaluated in a semi-quantitative fashion (0=no staining, 1=less than 50% of the tumor, 2= more than 50% of the tumor).

Data Collection and Analysis

Data on known prognostic factors including tumor stage, Ki-67 positivity, vascular or perineural invasion, lymph node metastasis, presence of necrosis, and soft tissue extension were reviewed and recorded. The relation between SSTR-2 positivity and known prognostic factors was tested using chi-square test. A p value <0.05 was considered as an indication of statistical significance.

RESULTS

Demographical, Clinical and Follow-Up Data

The mean age of the patients was 54.4 ± 12.3 years (range: 29-85 y), and the female to male ratio was 1.41. Tumor diameter ranged between 5 to 150 mm, with a mean of 40 mm. The tumor involved the entire pancreas (multifocal) in 83% of the cases, and was confined to the pancreas head in 7% and to the tail in 10%. Staging of the patients at the time of operation was as follows: stage 1, 24%; stage 2, 40%; and stage 3, 36%. All of the 41 patients were nonfunctional.

Patients were followed for a mean duration of 5.8 years. An early complication developed in 8 (19.5%) patients: 6 surgical site infections, 1 upper gastrointestinal bleeding, and 1 intraabdominal abscess formation. Only 1 out of 41 patients (2.4%) died in the early postoperative period, which was secondary to the development of atrial fibrillation.

The Relation between SSTR-2 Positivity and Known Prognostic Factors

Sixty percent of the patients had histologically confirmed SSTR-2 positivity, with 45% exhibiting focal (Figure 1) and 15% showing diffuse (Figure 2) staining characteristic.

Of 18 patients with SRS available, 3 (16.7%) had stage 3 disease based on WHO classification, whereas the corresponding figure was 39.1% ($n=9$) for the patients without SRS. Ki-67 was positive in 8 (44.4%) of the patients with SRS and in 10 (43.5%) of the patients without SRS.

Statistical analysis did not reveal a significant association between SSTR-2 positivity and any of the known prognostic factors for GEP NET: versus stage, $p=0.67$; vs. lymph node metastasis, $p=0.51$; vs. vascular invasion, $p=0.11$; vs. extension to surrounding soft tissue, $p=0.54$; vs. necrosis, $p=0.23$; vs. lymphatic invasion, $p=0.25$; and vs. perineural invasion, $p=0.42$.

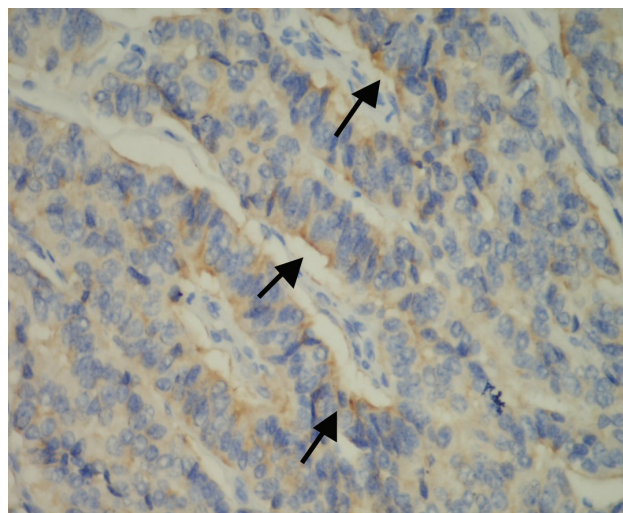


Figure 1. Focal membranous positivity in the SSTR-2 is monitored in the form of brown staining, which is marked with arrows, in a case with well-differentiated neuroendocrine carcinoma (Diaminobenzidine [DAB], X400).

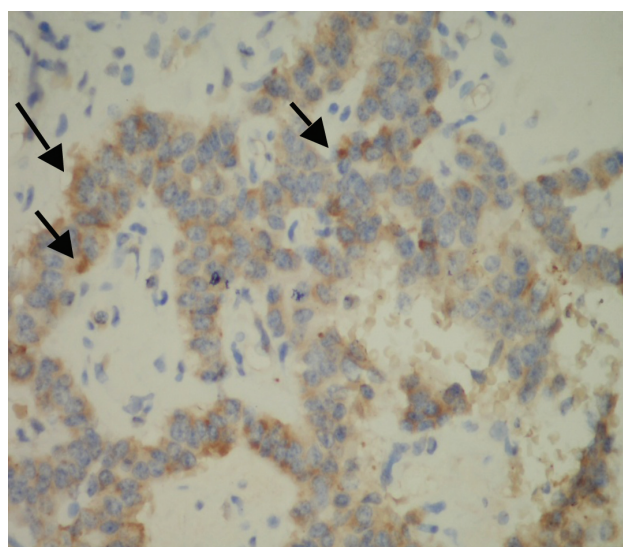


Figure 2. Diffuse cytoplasmic positivity in the SSTR-2 is monitored in the form of brown staining, which is marked with arrows, in a case with a well-differentiated neuroendocrine carcinoma (Diaminobenzidine [DAB], X400).

DISCUSSION

Identification of the SSTRs in GEP NETs represents a major advance in the management of these tumors. Although several subtypes of SSTR are expressed in endocrine tumors of the pancreas and gastrointestinal system, SSTR-2 is the most prominent type in more than 80% of these tumors (3,4), allowing the demonstration of GEP NETs with SRS and utilization of somatostatin analogues for adjunctive treatment (6-8). The synthetic so-

matostatin analogue octreotide is currently the most commonly used agent in scintigraphic evaluation of GEP NETs due to its strong binding to SSTR-2 and high level of SSTR-2 expression in these tumors. In addition, somatostatin analogues are used in their adjunctive treatment. Although the expression level of this receptor subtype predicts the response to somatostatin analogues, its prognostic role is still unclear. This study examined the prognostic role of SSTR-2 through testing its association with well-known prognostic factors and was unable to demonstrate any statistically significant relationship.

Long-acting synthetic somatostatin analogues like octreotide or octreotide-LAR inhibit the secretion of hormones responsible for clinical symptoms through binding SSTRs on NET cells (9). As a result, they provide symptomatic improvement and reduction in urinary 5-HIAA (5-hydroxyindoleacetic acid) levels in more than 90% of the cases (6). Somatostatin receptor analogues (octreotide, pentetreotide, lanreotide) have antiproliferative and antimetabolic activity through binding receptor subtypes of SSTR-1, SSTR-2, and SSTR-5 (10). They activate tyrosine phosphatase and alter intracellular calcium metabolism. The clinical benefit of somatostatin analogues is related to the presence of somatostatin receptors on tumor cells (11,12). Although complete regression of the tumor is rare, partial regression in tumor size is obtained during adjunctive treatment with somatostatin analogues. Currently, they represent the most effective palliative treatment modality in GEP NETs (13,14). The main problem associated with somatostatin analogues is tachyphylaxis.

Gastroenteropancreatic (GEP) NETs are slow-growing tumors. They are more amenable to resection and associated with better survival when compared to pancreatic adenocarcinoma (15). Surgery is the primary treatment for localized tumors and might be curative, giving five-year survival rates of 80–100% (16). Besides previously known predictive factors, other potential clinical and histopathological factors merit investigation. Since the disease is relatively rare, identification of the prognostic factors and development of an evidence-based staging system is rather challenging. Although a histopathological classification system developed by WHO currently exists, its clinical relevance is questionable (17).

Ki-67 positivity, lymph node metastasis, vascular or perineural invasion, presence of necrosis, and soft tissue extension were used in this study to

test their relation with SSTR-2 positivity, either focal or diffuse, since these are all known indicators of GEP NET prognosis. However, the lack of any relation between these parameters suggests that SSTR-2 receptor positivity does not predict prognosis, rather, it only seems to support the diagnosis of GEP NET. Nevertheless, SSTR-2 positivity, either focal or diffuse, may still have a prognostic value after octreotide treatment, similar to its predictive role for the treatment response to this receptor-specific agent (18). However, long-term studies with larger sample size and robust methodology are warranted to test this hypothesis.

Ki-67 positivity is important in GEP NETs since it is a proliferation index (19–21). Ki-67 is a high molecular weight nuclear protein antigen suggested to have an important role in cellular proliferation owing to its chromatin-related structure. Ki-67 proliferation index is related to the percentage of cells immunohistochemically positive for this antigen. Ki-67 positivity is associated with more rapid tumor growth and worse prognosis (22). In this study, 44.4% of the patients were positive for Ki-67; however, positivity for this antigen had no statistical relation with SSTR-2 positivity.

This study has several limitations. It has a retrospective design without a standardized management protocol. In addition, as is always the case in cancer-screening studies, some data are missing due to patients who are lost to follow-up. Since patients had scintigraphy at different time points in the course of the disease, it is not easy to comment on whether scintigraphy is beneficial or results in unnecessary loss of time and resources. This issue needs to be standardized, and another arm of this study is examining the role and timing of scintigraphy in this patient group.

In conclusion, SSTR-2 positivity, either focal or diffuse, had no relation with the known prognostic indicators in GEP NET patients, suggesting that it has no role in predicting survival rates after curative treatment. Considering the findings of previous studies with high level of evidence, we believe that somatostatin agonists should be used in adjunctive treatment of these patients. Many other factors may affect survival, including expression of other subtypes of SSTRs. This study evaluated the prognostic role of only SSTR-2, since it is known to be the most important subtype; however, studies on other subtypes would shed light on the prognostic role of this receptor group in the prognosis of GEP NET patients.

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