# Clinical and Pathological Features of Non-Functional Neuroendocrine Tumors of Pancreas: A Report from Iran

Neda Nozari[1], Sepideh Nikfam[1], Arash Nikmanesh[2], Mehdi Mohammadnejad[1], Rasoul Sotoudehmanesh[1], Farhad Zamani[3] ,Shahin Merat[1], Reza Malekzadeh[1], Akram Pourshams[1,*]

1. Liver and Pancreatobiliary Diseases Research Center, Digestive Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Pathology, Masood Clinic, Tehran, Iran
3. Gastroenterology and Liver Disease Research Center, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran

* **Corresponding Author:**
Akram Pourshams, MD, MPH
Digestive Disease Research Institute, Shariati Hospital, N Kargar St. Tehran14117, Iran
Tel: + 98 21 82415104
Fax:+ 98 21 82415400
Email: akrampourshams@gmail.com
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## ABSTRACT

### BACKGROUND

Pancreatic neuroendocrine tumors (PNETs) are rare tumors with variable malignant potential, prognosis, and survival. We aimed to assess the characteristics of patients with non- functional PNET in our hospital.

### METHODS

From Nov 2010 to Nov 2013 , all patients who came to endosonography unit of Shariati hospital , Tehran , Iran , and had pancreatic lesions were assessed . Tumor samples were obtained through fine needle aspiration. Various characteristics of the non- functional PNET were recorded and patients were followed up to three years.

### RESULTS

Twenty eight non func-PNET cases, aged 37-72 years were identified, 15 (53.6%) of whom were men. Fifteen (53.6%) tumors were located in the head and 5(17.8%) in the body of the pancreas. The mean tumor size was 3.9 Cm and 10.7% , 28.6%, 32.1%, and 28.6% of the patients were at stages I, II, III and IV, respectively. Of the patients, 12 (43%) underwent surgery, 3 (10.7%) received chemotherapy, and 13 (46.4%) received no treatment. During the mean follow-up of 16 months, the disease had progressed in 3 (10.7%) patients and 10 (35.7%) had died. In univariate analysis, tumor size>3Cm and Ki-67>20% were correlated with survival rate but not in multivariate analysis.

### CONCLUSION

Iranian patients with non- functional PNET present similar characteristics to world patients. There is a need to establish efficacy of tumor samples which are obtaining through fine needle aspiration for assessing tumor grading.

KEYWORDS
Neuroendocrine Tumors; Epidemiology; Survival

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## INTRODUCTION

Neuroendocrine tumors (NETs) are rare tumors[1,2] and only 12% of gastroenteropancreatic NETs are found in the pancreas (PNET).[3] PNETs are more aggressive than NETs in other parts of the gastrointestinal tract.[3,4] The peak prevalence for PNET is in 30-60 year-olds with

no sex predilection.[5] 50% of PNETs are located in the head, 26.9% in the body, and 23.1% in the tail of the pancreas.[6]

PNETs produce various symptoms and may or may not be functional.[1-3] These tumors have different rates of growth, malignant potential, prognosis, and survival.[2] Functional tumors often present with symptoms of gastrinoma or insulinoma, while non-functional tumors are usually found incidentally or present with symptoms of mass effect or are discovered after metastasis in very advanced stages.[1,2,5] The 5-year survival rates vary from 41% to 95%.[3] The diagnosis of PNETs is usually delayed for up to 5-7 years after onset of clinical symptoms and this delay often leads to a significant decrease in survival.[3]

Imaging methods such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and somatostatin receptor scintigraphy (SRS) are used to diagnose and localize PNETs.[1] Endoscopic ultrasonography (EUS) is especially useful in localizing, staging, and confirming the diagnosis.[1,3] Fine needle aspiration (FNA) by EUS is the most commonly used technique for confirming the diagnosis of NET. The sensitivity of EUS for detecting PNETS <2cm is 87%.[1]

The proliferation index can be assessed by counting the number of mitosis per high power field on a hematoxylin and eosin stained slide or by counting positively stained cells with Ki-67 antibody. The Ki67 labeling index is widely used as an aid in grading NETs: G1 (Ki-67 ≤2%), G2 (Ki-67= 3-20%), and G3 (Ki67>20%).[3]

After confirming the diagnosis, patients in earlier stages (I and II) can be treated by either complete tumor resection or pancreatectomy with lymphadenectomy. Tumor resection is considered a curative treatment.[2,3,7] The first line of treatment is chemotherapy in high grade PNETs.[7] Liver transplantation might be indicated when liver metastasis exists without extrahepatic lesions, although transarterial chemoembolization and radiofrequency ablation (RFA) may also help.[2,3]

## MATERIALS AND METHODS

Shariati Hospital is a tertiary referral center affiliated to Tehran University of Medical Sciences, Tehran, Iran. Patients with pancreatic mass identified during EUS in Shariati Hospital during November 2010-November 2013 were selected. The clinical and laboratory data of these patients including sex, age, clinical symptoms, laboratory data, tumor size and location, endocrine function, distant metastasis, lymphadenopathy, and treatment plan were recorded in Digestive Disease Research Institute.

Patients exhibiting signs and symptoms attributable to tumor-secreted hormones (often are with gastrinoma or insulinoma symptoms) were classified as functional PNETs. Non-functional tumors were defined as being asymptomatic or had no signs, which were induced by tumor hormone. FNA was performed in all non-functional PNETs (NF-PNETs) by three experienced gastroenterologists and examined by two expert pathologists. The endosonographer inserted a 22 gauge needle into the target lesion and aspirated samples at least three times almost without applying suction. Lesions in the pancreatic head or uncinate process were aspirated through the transduodenal approach and lesions in the body and tail of the pancreas through the transgastric route. All samples were sufficient for cytologic evaluation and there was no need to repeat FNA in any patient. In patients for whom pancreatic resection was performed, the surgical specimen was sent for pathological analysis.

Diagnosis of PNET was confirmed by two expert pathologists using immunohistochemical (IHC) evaluation for cytokeratin, CD5, CD20, leukocyte common antigen, chromogranin A, and synaptophysin on all samples obtained by FNA and surgical resection.

If IHC stain was positive for chromogranin A or synaptophysin then the Ki67 labeling index was calculated using mouse monoclonal ki-67 antibody (clone MIB-1, Dako, glostrup, Denmark).[8] Then, pathological classifications were completed based on the 2010 WHO classification.[3,9] Tumor staging was done based on TNM classification of the European Neuroendocrine Tumor Society.[9]

Surgical resection was recommended for PNETs grade I and II and chemotherapy for grade III and IV. Patients were allowed not to receive any treatment if they chose so.

The primary end points were overall survival (OS) and progression-free survival (PFS). OS is defined as either the length of time since diagnosis until death or until the last follow-up. PFS, in the case of patients undergoing operation, is the time from resection to recurrence. For patients not operated, the PFS is defined as the time from diagnosis to documented increase in stage, which is in fact the length of time that a patient lives with PNETs but the disease is worsening.

The patients were visited every six months and CT or MRI of pancreas was performed. If recurrence was suspected, histological confirmation was obtained.

**Data analysis and statistical considerations:**

Analysis was done using SPSS software, version 19. $p<0.05$ was considered significant. Kaplan-Meier method was used for analysis of OS and PFS. Cox regression method was used for multivariate analysis.

The study protocol was approved by the Ethics Committee and Institutional Review Board of the Digestive Disease Research Institute of Tehran University of Medical Sciences, Tehran Iran.

## RESULTS

A total of 28 patients, with a mean age of 53.5±9 years (range: 37-72 years) were confirmed to have non-functional PNETs. Of them 15 (53.6%) were men. The average time between onset of symptoms to establishing the diagnosis was 4.4 months. The predominant presenting symptoms included abdominal pain (n=21, 75%) and weight loss (n=15, 53.5 %). Other symptoms like jaundice, pruritus, fever, urine discoloration, early satiety, stool discoloration, shaking chills, nausea, and vomiting were rare. The mean tumor size was 3.9±1.96 cm (range: 1.4-8.5 cm). Local infiltration including adjacent organs such as duodenum and large vessels was found in 14 (50%) patients. Distant metastasis was seen in 8 (28.5%) patients. The synaptophysin stain was positive in all the patients, while chromogranine A stain was positive in only 17 (60.7%) patients. The mean Ki-67 labeling index was 11.25% (1-80%).

Details of the clinical characteristics of the patients are given in table 1. Twelve (42.8%) patients underwent only surgery, 7 (25%) received surgical treatment followed by chemotherapy, 3 (10.7%) patients received only chemotherapy (oxaliplatin-containing regimens), and 13 (46.4%) patients received no treatment. During a mean follow-up of 16 months (range: 1-36 months), the disease had progressed in 3 (10.7%) patients and 10 (35.7%) had died (figure 1).

The following factors were analyzed to detect their effect on the survival: sex, age, tumor site and size, solid lesion, presence of lymphadenopathy, distant metastasis, UICC stage, and tumor grade (Ki67 index). In univariate analysis, parameters with statistically significant effect on OS were: tumor size>3Cm (HR=5.8, 95% CI: 1.17- 28.75) and Ki-67>20% (HR=5.9, 95% CI: 1.16 -19.37). None were found to be significant in multivariate analysis.

We could not calculate the median PFS rate because we had too few cases with recurrence or change in tumor stage. Only three patients had postsurgical recurrence and no patient had change in stage. A univariate analysis of sex, age, tumor site, tumor size, solid vs. cystic, presence of lymphadenopathy, distant metastasis, UICC stage, tumor grade, and Ki-67 labeling index did not show any significant effects on PFS. The OS and PFS during follow-up are shown in table 2.

## DISCUSSION

Although NETs are still rare but they have an increasing trend. The incidence of NETs increased from 1.1/100 000 per year in 1973 to 5.3/100 000 per year in 2004 in a North American Surveillance[10] (almost 0.2-0.3/100000 for PNETs)[11] which is partly attributed to the increased awareness of clinicians and pathologists as well as development and improvement in diagnostic tools such as immunohistochemistry (IHC) staining for chromogranin A and imaging tools. However a real increase in oc-

**Table 1:** Clinical characteristics of the studied patients

| | | Patients' number |
|---|---|---|
| **Gender** | Female | 13 (46.4%) |
| | Male | 15 (53.6%) |
| **Blood group** | O + | 12 (42.8%) |
| | A + | 7 (25%) |
| | B + | 7 (25%) |
| | AB + | 2 (7.2%) |
| **Tumor location** | Head | 15 (53.6%) |
| | Body | 5 (17.8%) |
| | Head-Body | 2 (7.15%) |
| | Body-Tail | 2 (7.15%) |
| | Tail | 4 (14.3%) |
| **Lesion type** | Solid lesions | 21(75%) |
| | Solid - cystic | 6(21.4%) |
| **Cystic lesion** | | 1 (3.6%) |
| **Grade** | 1 | 17(60.7%) |
| | 2 | 7(25%) |
| | 3 | 4(14.3%) |
| **Distant metastasis** | | 8(28.5%) |
| **Liver** | | 7(25%) |
| **Peritoneum** | | 1(3.5%) |
| **Lymhadenopathy** | | 16(57.1%) |
| **Stage** | I | 3(10.7%) |
| | II | 8(28.5%) |
| | III | 9(32.2%) |
| | IV | 8(28.6%) |

**Table 2:** Overall survival (OS) and progression-free survival( PFS)

| | Cumulative survival rates (95% CI) | |
|---|---|---|
| | **PFS** | **OS** |
| 1 year | 0.65 (0.43-0.80) | 0.76 (0.55-0.89) |
| 2 year | 0.48 (0.27-0.67) | 0.61 (0.37-0.78) |
| 3 year | 0.36 (0.13-0.61) | 0.48 (0.21-0.71) |

PFS: Progression-free survival, OS: Overall survival

currence is also likely.[10]

PNETs account for 1–2% of all pancreatic neoplasms and NF-PNETs constitute about 65% of pancreatic neuroendocrine tumors.[12] Because NF-PNETs are rare, information on their natural history and prognostic factors is sparse. This study represents the first clinicopathological description regardingNF-PNETs in an Iranian population from

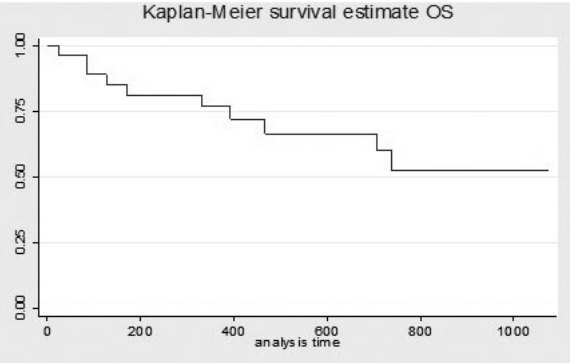


**Fig. 1:** Overall survival in patients with PNETs during 1074 days follow-up

a reputable center for EUS. Characteristics such as mean age at diagnosis, almost equal distribution between both sexes, head of pancreas as the main location for the tumors, symptoms and signs, stage at presentation, overall survival, and prognostic indicators of our patients are comparable with previous reports.[13]

As far as we found in our thorough literature review, this is the first prospective study in which biomarker investigation and proliferation index were assessed successfully on fine needle aspiration biopsy despite surgical pathological samples.

In Nordic Guidelines (2010) for the diagnosis and treatment of gastroenteropancreatic NETs, it is emphasized that formalin-fixed paraffin-embedded 1.2 mm biopsies or surgical specimens are needed for diagnosis and fine needle aspiration biopsy is not recommended since it is unlikely to give a definite diagnosis and will not yield optimal material for IHC and it is impossible to evaluate proliferation index by Ki67 on fine needle aspiration.[14]

Our success can be attributed to the excellent job of our endosonographer for yielding adequate tumoral cells through fine needle aspiration and our pathologists for their thorough investigation of the tumor cells.

In a recent published retrospective study (1992-2010) from the United States, 132 cases were diagnosed with PNETs by EUS-guided FNA. Histological correlation was available for 58% of the FNAs. Only 54 of the 70 histologically confirmed cases of PNETs (77%) were correctly diagnosed by preop-

erative FNA. Diagnostic pitfalls in the study mainly included ductal adenocarcinoma, pseudo-papillary tumor, and chronic pancreatitis.[15]

In our study, 19(67.85%) out of the 28 patients had surgical specimens, all of whom had histologically confirmed PNETs. It is recommended to perform multicenter prospective studies with more NF-PNETs cases for reaching a better conclusion.

The weakness of our study is not having the results of biochemical evaluations, especially serum chromogranin A levels to compare our findings with other studies.[16]

Positive rate of synaptophysin (100%) and chromogranin A (60%) in our FNA samples is somewhat different from positive rates in China (chromogranin A and synaptophysin were 81.1% and 87.7%, respectively). It could be partially because of having different pathological sources, FNA versus surgical specimen, and organs. In the previously mentioned study in China, the authors reported the results for all gastroenteropancreatic neuroendocrine neoplasms.[17]

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## CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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