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ORIGINAL ARTICLE

Clinicopathological characterization of gastroenteropancreatic neu-roendocrine neoplasms: a retrospective study of 48 cases

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Abstract

Objective Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) constitute a rare and heterogeneous group of tumors with varied biology and still constitute a diagnostic and therapeutic challenge for physicians of all specialties. In the present study, we aimed to review and study the clinicopathological characteristics of GEP-NENs applying the World Health Organization (WHO) 2010 grading criterion.

Methods A total of 48 patients were enrolled in the study. The study included patients diagnosed with GEP-NENs who were treated and followed up at our Hospital from January 2013 to December 2017. Data regarding clinicopathological features of the patients were retrospectively evaluated. The expression of neuroendocrine markers was measured using the immunohistochemical *Ultra Sensitive™ S-P* method of staining in 48 cases of primary GEP-NENs; and serum levels of neuron-specific enolase, carbohydrate an-tigen 19-9, and carcinoembryonic antigen in 36 GEP-NEN patients were measured using the electrochemiluminescence method.

Results The median age at presentation was 59.3 (range 48–82) years, and 39 cases (81.3%) were seen between the 5th and 6th decades. There was a male predilection (male: female=3:1). In 79.2% cases (38/48), tumors were hormonally nonfunctional. The most common presentation was abdominal pain, and the most frequent primary site of the tumor was the rectum, followed by the stomach (n = 15, 31.3%), colon (n = 5, 10.4%), and so on. Of the 48 tumors, 16 (33.3%) were G1, 6 (12.5%) cases were G2, 16 (33.3%) were neuroendocrine carcinoma (NEC), and 10 (20.8%) were mixed adenoneuroendocrine carcinoma (MANEC). According to the AJCC/UICC classification, 45.8% (n = 22) were diagnosed at low stage (stage I or II) while 54.2% (n = 26) were diagnosed at high stage (stage III or IV) (the majority of NEC, G3, and MANEC). A male preponderance was noted for all tumors except for G2 neoplasms, which showed no gender predilection. Thirty-nine patients underwent endoscopic biopsy. The lesions in 18.8% (n = 9) of the patients were indentified only radiologically. After the surgical procedures, 36 had at least one follow-up visit with a median follow-up duration of 5 months; the mean follow-up period was 28 ± 16 months. The one-year and three-year survival rates were 72.2% (26/36) and 61.1% (22/36), respectively. This study did not find an effect of grade 3 (G3) of tumor on the short-term clinical outcome of these patients. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC classification (P < 0.05), and metastases at diagnosis (P < 0.05) were associated with poorer prognosis.

Conclusion Most GEP-NENs are nonfunctional and nonspecific in presentation. The most frequent primary site of the tumor was the rectum and the commonest ages at diagnosis were the 5th and 6th decades. Endoscopic biopsy is the main diagnostic and histological grading method for GEP-NEN. In the survival analysis, NEN G3, a higher stage according to the AJCC/UICC classification, and metastases at diagnosis are associated with poorer prognosis.

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Key words: Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs); Ki 67/MIB-1 index; mitotic rate; diagnosis; prognosis

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Neuroendocrine neoplasms (NENs) are tumors arising from the neuroendocrine cells which are distributed throughout the body. Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) were originally identified as rare diseases occurring in the gastrointestinal tract and pancreas and displaying distinctive histopathological features from those of conventional gastroenteropancreat-ic epithelial cancers [1–2]. GEP-NENs refer to a group of heterogeneous cancers of neuroendocrine cell phenotype that mainly fall into one of two subtypes: gastroenteropancreatic neuroendocrine tumors (GEP-NETs) or gastroenteropancreatic neuroendocrine carcinomas (GEP-NECs), and are a highly heterogeneous and poorly understood group of rare but increasingly prevalent tumors with varied clinical presentation [3–4]. Most GEP-NENs, however, are nonfunctional and have non-specific presentations, which makes their early diagnosis challenging [1, 3]. They still constitute a diagnostic and therapeutic challenge for physicians of all specialties [1–2, 4–5].

Materials and methods

Diagnostic criteria for GEP-NENs

According to the World Health Organization (WHO) 2010 classification, GEP-NENs are classi-fied as NET Grade 1 (G1) and NET Grade 2 (G2) (well-differentiated endocrine tumors), and NEC Grade 3 (G3) (poorly differentiated endocrine carcinoma) [1]. The WHO 2010 classification takes into account the mitotic rate (usually expressed as mitoses per 10 high power microscopic fields or per 2 mm) and/or Ki-67 index (the percentage of neoplastic cells immunolabeled for the proliferation marker Ki-67) when grading GEP-NENs. Tumors with a Ki-67 index of < 2% or a mitotic rate of < 2/10 HPF are classified as G1, those with a Ki-67 index of 3–20% or a mitotic rate of 2–10/10 HPF are classified as G2, and those with a Ki-67 index of > 20% or a mitotic rate of > 20/10 HPF are classified as G3 [6–7] (Table 1).

Patients

This study included all cases of GEP-NEN involving the stomach, duodenum, jejunum, ileum, appendix, colon, rectum, and pancreas that were treated and followed up at our hospitals from January 2013 to December 2017. A total of 48 cases were enrolled in the study; among them, there were 39 patients from Rizhao People's Hospital, 5 from Rizhao Lanshan District People's Hospital, and 4 from Weihaiwei People's Hospital. The expression of neuroendocrine markers and Ki-67 was measured using the immunohistochemical *Ultra Sensitive™ S-P* method of staining in 48 cases of primary GEP-NENs; and the levels of neuron-specific enolase (NSE), carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA) in 36 cases of gastrointestinal neu-roendocrine neoplasm patients were measured using the electrochemiluminescence method. All data regarding clinicopathological features and follow-up information were reviewed and evalu-ated. Of the 48 cases, 39 cases included endoscopic biopsies, and 48 cases had resection speci-mens. Based on WHO 2010 classification of GEP-NENs, all cases were graded as G1, G2 or G3. GEP-NENs mainly fall into one of two subtypes: well-differentiated GEP-NETs, or poorly dif-ferentiated GEP-NECs, and mixed adenoneuroendocrine carcinoma (MANECs). All the clinical and follow-up information were reviewed and evaluated, and their relationship with well-known clinicopathological factors such as tumor size, grade, lymph node status, and stage were investigated in GEP-NETs patients. The patients diagnosed with GEP-NETs had not been treated with hormone endocrine therapy, anti-neoplastic chemotherapy or radiotherapy during the pre-ceding six months. The follow-up details which were available until the end of the study period were collected. Permission was obtained from the local ethical committee to collect GEP-NET tissues and all patients signed informed consent forms prior to enrolment in the study.

Pathologic study

In this study, pathological diagnoses were made after histological staining of surgically resected or endoscopically biopsied tumor samples, and independently verified histologically by two pathologists, and pathological categorization was determined according to the current WHO clas-sification system diagnostic criteria (2010) [1]. The histopathological features and immunohisto-chemistry

Table 1 WHO 2010 classification of GEP-NETs

Grade	Two grade categories equivalent in WHO classification, 2010	Ki 67/MIB-1 index (%)	Mitotic rate (/10 HPF)
NET Grade 1	Well-differentiated endocrine tumors	< 2	< 2/10 HPF
NET Grade 2		3–20	2–20/10 HPF
NEC Grade 3 or MANC Grade 3	Poorly differentiated endocrine carcinoma	> 20	> 20/10 HPF

Note: NET: neuroendocrine tumor; NEC: neuroendocrine carcinoma; MANEC, mixed adenoneuroendocrine carcinoma; HPF, high power fields

details of all 48 cases were analyzed. The expression of neuroendocrine markers, such as CD56, chromogranin A (CgA), synaptophysin (Syn), NSE, cytokeratin (CK) 7, and Ki-67 were measured by the immunohistochemical method in 48 cases of primary GEP-NETs, 48 cases of dysplasia tissue closely adjacent to carcinomas, and 40 cases of normal colorectal mucosal specimens with complete clinical data from 2013 to 2017. All specimens were fixed in formalin and embedded in paraffin. Serial sections (4 μm) were deparaffinized in xylene and hydrated through a graded series of ethanol. The specimens were washed in phosphate-buffered saline within five minutes and examined under a binocular dissecting microscope. Immunoreactions were processed using the Ultra Sensitive™ S-P kit (Maixin-Bio, China) according to the manufacturer's instructions, and signals were visualized using the 3, 3'-diaminobenzidine substrate, which stains the target protein yellow. Negative controls were used. The primary antibody was replaced with phosphate-buffered saline, containing 0.1% bovine serum albumin of the same concentration as the primary antibody. The positive controls were tissues known to express the antigen being studied. CD56, CgA, Syn, NSE, CK7, and Ki-67 immunoreactivity expression was evaluated as the percentage of cancer cells that showed cytoplasmic staining reactivity. For Ki-67 expression, the percentage of cancer cells showing nuclear reactivity was recorded after inspection of all optical fields at 200× power and the mean value was used to score each case. Assessment of the staining was evaluated by two independent pathologists blinded to the clinical statuses of the patients.

Measurement of biomarkers in serum

The serum concentrations of NSE, CA 19-9 and CEA were measured using the electrochemilu-minescence immunoassay from Roche according to the manufacturer's instructions (Roche Diag-nostics, Germany). Three milliliters of blood was drawn from each patient and heparinized. The biomarker levels were detected in 36 cases of GEP-NET using the electrochemilu-minescence method in the clinical laboratory of Rizhao People's Hospital. The cut-off values of NSE, CA 19-9 and CEA in serum are 16.3 ng/mL, 27 U/mL and 3.40 ng/mL, respectively. For the biomarker levels, patients are divided into two groups (normal level or high-level peripheral blood). Serum levels of NSE, CA 19-9, and CEA above 30 ng/mL, 27 U/mL and 5 ng/mL, respec-tively, were considered as significantly elevated. In the case of multiple measurements, the high-est level was reported. The expression of Ki-67 proliferation index; the levels of NSE, CA 19-9 and CEA; and histological grade, regional lymph node metastasis, distant metastasis and recur-rence on record were also assessed in order to study the clinical and pathological characteristics associated with GEP-NETs.

Statistical analysis

Measurement data expressed as the mean and standard deviation (mean ± SD) between groups were compared using the *t*-test, while categorical data were compared using the chi-square (χ^2) test. *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 17.0 (SPSS Inc., USA).

Results

Clinical features

There were 36 male cases and 12 female; overall, there was male predilection (male: female = 3:1) in this study. Grade 1 tumors showed no male predilection (male: female = 9:7) as compared to the other grades (male: female = 27:5). The median age at presentation was 59.3 (range 48–82) years. Thirty-nine cases (81.3%) were seen between the 5th and 6th decades. The study patients had a delay of 2 (0–16) months from their first symptoms to their final diagnosis at the hospital. In 38 cases (79.2%), the tumors were hormonally nonfunctional. The most common presentation was abdominal pain, which was seen in 68.8% (33/48) of patients, followed by altered bowel habits (14/48, 29.2%), loss of weight and appetite (13/48, 27.0%), and abdominal mass (5, 10.4%). The most frequent primary site of the tumor was the rectum (n = 20, 41.7%), followed by the stomach (n = 15, 31.3%), colon (n = 5, 10.4%), pancreas (n = 4, 9.5%), small intestine (n = 3, 6.25%), and appendix (n = 1, 2.1%). These data were shown in Table 2.

Serum concentrations of biomarkers

The serum concentrations of CEA, NSE, and CA 19-9 are shown in Table 3. In the case of multi-ple measurements, the highest level was reported. The serum NSE and CEA levels were signifi-cantly higher in the poorly differentiated GEP-NEN groups than the well-differentiated groups (both $P < 0.05$), and the serum CA 19-9 levels were not significantly different between the groups (both $P > 0.05$). There were no significant differences in CEA, NSE and CA 19-9 levels between the GEP-NEN G1 and G2 groups; there were also no significant differences between the GEP-NEN NEC and MANEC groups (both $P > 0.05$). Compared with the group with Ki-67 index less than twenty percent, the serum levels of CEA, NSE, and CA 19-9 were significantly higher in the group with Ki-67 index more than twenty percent ($P < 0.05$).

Endoscopic and radiological findings

Among the 48 patients, 39 underwent endoscopy and so had available findings. In 39 (81.3%) patients, the

Table 2 Clinical characteristics of the GEP-NEN cases in the study group (n = 48)

Characteristic	n
Gender	
female	36
male	12
Age at diagnosis	59. 3 (48–82)
< 50 years	2
5th decade	19
6th decade	20
> 60 years	7
Hormonal activity	
Nonfunctioning NEN	38
Functioning NEN	10
Diagnosis method	
Endoscopy	39
CT	9
Primary tumor site	
stomach	15
small intestine	3
colon	5
rectum	20
appendix	1
pancreas	4
Grade (WHO classification, 2010)	
NEN G1	16
NEN G2	6
NEC G3	26
NEC	16
MANEC	10
AJCC/UICC classification	
Low stage (I or II)	22
High stage (III or IV)	26

Note: NEC, neuroendocrine cancer; NEN,neuroendocrine neo-plasm; AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control; CT, computed tomograph

Table 3 Serum biomarker levels in the GEP-NEN cases in the study group (n = 36)

Grade	n	NSE ng/mL	A8 U/mL	CEA ng/mL
Well-differentiated	13			
NET G1	9	63. 7 ± 27. 2	37. 8 ± 21. 2	16. 9 ± 7. 9
NET G2	4	84. 3 ± 32. 9	39. 3 ± 23. 4	29. 8 ± 14. 2
Poorly differentiated	23			
NEC	15	98. 8 ± 48. 6	39. 7 ± 22. 9	42. 8 ± 19. 8
MANEC	8	122. 8 ± 75. 3	42. 3 ± 28. 7	53. 3 ± 22. 6

primary site was identified by endoscopic biopsy; in the remaining 9 (18.8%) patients, probable primary lesions were identified on radiological examination alone. Computed tomography (CT) scan showed a single mass 0.6–10.7 cm in maximum dimension; the largest lobulated mass 10.7 cm in maximum dimension was identified in the abdominal pancreas. CT scan showed that 2 patients had local mucosal destruction of the digestive tract wall which was interrupted, 2 patients had unevenly thickened lesions, 2 patients had annular thickened lesions, and in 3 patients the serous surface was clear with no tumor involvement. Upon enhancement, 2 patients had obvious enhanced lesions; enlarged lymph nodes could be seen in 4 patients (Fig. 1).

Pathological findings

Gross examination

Of the 48 study samples, 36 were resection samples available for gross examination and the re-maining 12 were endoscopic biopsy samples. Of the 36 cases, the cut surface of the tumor in all resection specimens had a single tumor nodule, ranging in size from 0.6 cm to 10.7 cm in maxi-mum dimension with a soft grey-white to yellow cut surface. Focal areas of hemorrhage were seen in 3 cases; grey-white zones with focal areas of necrosis were seen in 4 cases. There was no evidence of gross vascular invasion. The surrounding tissue was normal.

Histopathology

Histologically, the low grade tumors (G1 and G2) had classical patterns of arrangement including nests (n = 23), cords (n = 12), trabeculae (n = 18), festoons (n = 22), ribbons (n = 10), sheets (n = 8), gyriform (n = 6), acinar (n = 6), and pseudopapillary (n = 3) patterns. The cells were round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei with uniformly dispersed coarse chromatin and inconspicuous mitotic activity (mitotic rate: 0–10/10 HPF). The high-grade tumors (GEP-NECs and G3) showed sheet and nest patterns. The cells were medium to large sized, polygonal, with scanty to moderate amounts of eosinophilic cytoplasm, with mild to moderately pleomorphic nuclei, and with finely dispersed chromatin. There was increased mitotic and apoptotic activity (mitotic rate: 11–56/10 HPF) in NECs. Ten cases of MANEC had unequal adenoid structure, morphology consistent with small cell carcinoma with sheets and nests of polygonal cells displaying moderate nuclear pleomorphism and increased mitotic and apoptotic activity (mitotic rate: 16–56/10 HPF) (Fig. 2).

Immunohistochemistry

Immunostaining for neuroendocrine markers (CD56, CgA, Syn, and NSE), CK7 and Ki-67 were carried out in all 48 cases. CgA was positive in 32 (66.7%), Syn positive in 37 (77.1%) cases, NSE in 29 (60.4%) cases, and CD56 in 37 (77.1%) cases. CK7 immunostaining was performed in 12 cases with poorly differentiated neoplasms and showed positive staining in adenoid structure with G3 tumor of MANEC. A mean Ki-67 proliferation index of 10% (range 0–19%) in well-differentiated endocrine

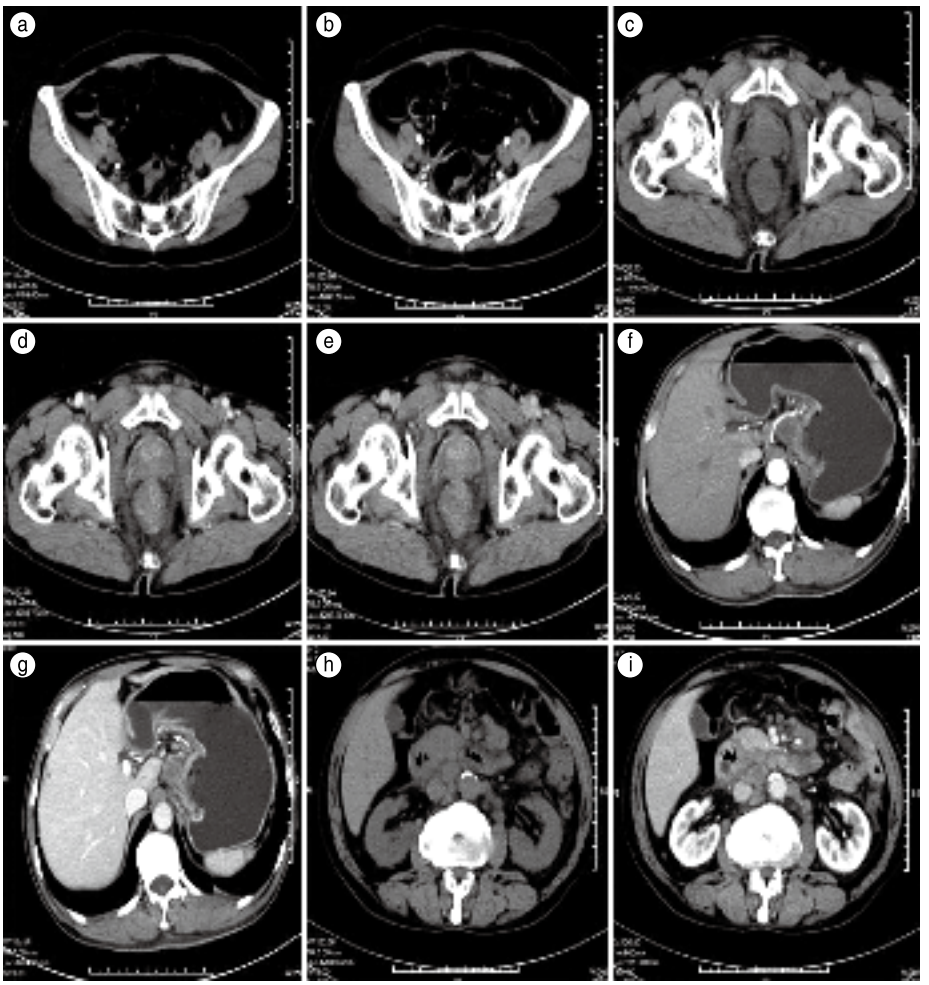

Fig. 1 NEN image findings. (a, b) sigmoid colon NET G1, the colon wall was locally thickened, obviously enhanced, and the serous surface was clear. (c–e) rectal NEC G3, the rectal wall was significantly annularly thickened and inhomogeneously enhanced with an indistinct serous surface. (f, g) stomach NEC G3, The gastric wall was thickened, the mucosa was interrupted, and enlarged lymph nodes were seen. (h, i) MANEC G3, the wall of the descending duodenal segment was thickened, with uneven thickness and obvious uneven enhancement.

tumors (WHO G1 and G2) and 25% (range 0–80%) in poorly differenti-ated endocrine carcinoma (WHO G3). Ten cases of MANEC had a mean Ki-67 proliferation index of 20% (range 10–70%) in the adenoid structure area and 50% (range 10–80%) in the endo-crine carcinoma area. The expression of Ki-67 in endocrine carcinoma and MANEC tissues was obviously higher than that in adjacent tissue and normal mucosal tissue (both $P < 0.05$). Ki-67 pro-liferation was significantly correlated with the medians of mitotic, and Ki-67 proliferation and the medians of mitotic were both significantly correlated with the grading (G3 vs G1, 2), stage and lymph node metastasis and distant metastasis (each $P < 0.05$) (Fig. 2).

Grade

Based on WHO 2010 grading of the 48 tumors, 16 (47.7%) were G1, 6 (12.5%) cases were G2, 16 (47.7%) were NECs, and 10 (20.8%) were MANECs, as WHO G3. According to the Ameri-can Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) classifica-tion, 45.8% ($n = 22$) were diagnosed at low stage (stage I or II), 54.2% ($n = 26$) were diagnosed at high stage (stage III or IV) (the majority of NEC G3 and MANEC). A male preponderance was noted in all tumors except for G2 neoplasms, which showed no gender predilection.

Follow-up

After the surgical procedures, 36 of the 48 patients had at least one follow-up visit with a median duration of follow-up of 5 months; the mean follow-up period was 28 ± 16 months. The one-year and three-year survival rates were determined to be 72.2% (26/36) and 61.1% (22/36), respective-ly. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC

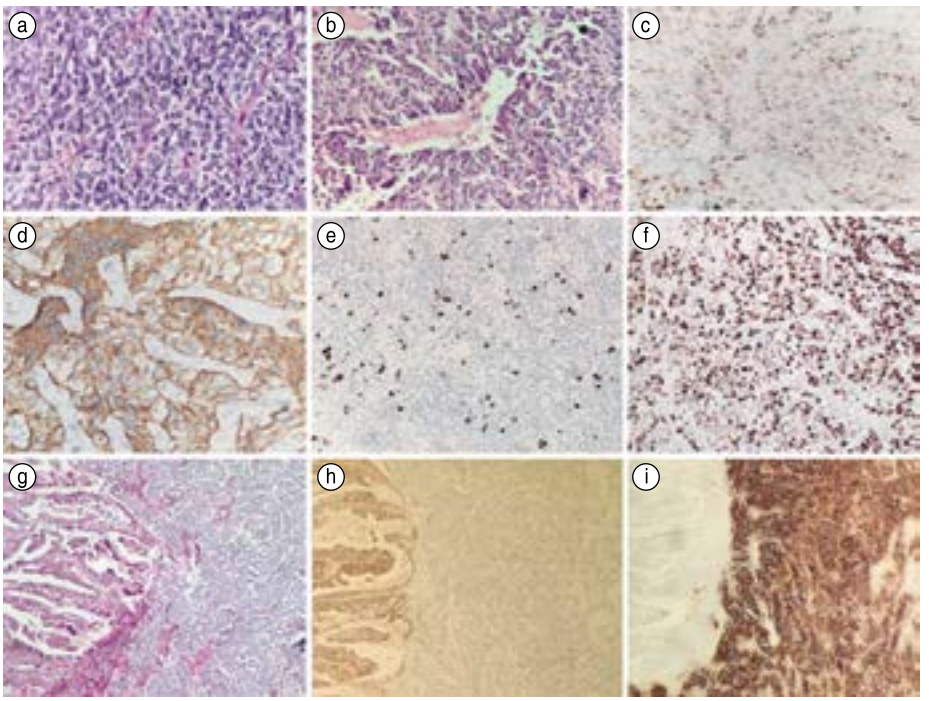

Fig. 2 NEN Histopathology and Immunohistochemistry. Histologically, the cells were round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei (a and b, HE stain). Immunostaining showed positive findings for neuroendocrine markers CgA (c) and Syn (d); Ki-67 proliferation index was less than 2% (e) and 70% (f) (Ultra SensitiveTM S-P stain); (g, i) MANEC G3, histological structure of mixed adenocarcinoma (left area) and neuroendocrine carcinoma (right area) (g, HE stain), CK7 positive in adenocarcinoma (h) and NSE positive in neuroendocrine carcinoma (i) (Ultra Sensitive™ S-P stain).

classification ($P < 0.05$), and metastases at diagnosis ($P < 0.05$) were associated with poorer prog-nosis. There was no significant correlation with sex, site, and age at diagnosis ($P > 0.05$).

Discussion

As mentioned, GEP-NENs are largely divided into GEP-NETs and GEP-NECs, according to the classification criteria defined by the WHO [1–2]. The annual global incidence of NEN has increased, with a fivefold increase over the past 30 years in the United States, possibly due to improvements in endoscopic cancer screening. This increase in the incidence of GEP-NENs has resulted in greater attention being paid to these diseases [1–2, 5]. In our study, there was a male predilection (male: female = 3:1). NET G 1 showed no male predilection (male: female = 9:7) as compared to the other grades (male: female = 5.4:1). The median age at presentation was 59.3 (range 48–82) years, and 81.3% were seen between the 5th and 6th decades. In our study, 79.2% of tumors were hormonally nonfunctional. However, the serum NSE and CEA levels were significantly higher in the poorly differentiated GEP-NEN groups than the well-differentiated groups, and the serum CA 19-9 levels were not significantly different between the groups. There were no significant differences in CEA, NSE and CA 19-9 levels between the GEP-NEN G1 and G2 groups, and there were also no significant differences between the GEP-NEN NEC and MANEC groups. In this study, the most common presentation was abdominal pain, which was seen in 68.8% of patients. In our study, the most frequent primary site of the tumor was the rectum, which is consistent with other reports [6], followed by the stomach, colon, pancreas, small intestine, and the appendix had the lowest incidence in our study group. The most common primary tumor site in most reports from Europe and the United States was the small intestine [1–2, 7–8]. However, in Asian epidemiological surveys, rectal NENs were more frequent [6].

A GEP-NEN diagnosis is based on the loss of epithelial tubular gland structures [9], the diffuse expression of neuroendocrine markers (particularly of CgA, Syn, and CD56) and the proliferative cell rate, as represented by the Ki-67 index and the mitotic count [1–2, 10–12]. In this study, histolog-ical structures such as festoons, nests, trabeculae, cords, ribbons, sheets, gyriform, acinar, and pseudopapillary were all seen. Pseudopapillary patterns were seen in 3 cases of pancreatic tumor. The cells were round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei with uniformly dispersed coarse

chromatin and inconspicuous mitotic activity. Our study group showed the high-grade tumors (GEP-NEC G3) with mainly sheets and nests patterns and the cells were medium to large sized, polygonal, with scanty to moderate amounts of eosinophilic cytoplasm, mild to moderately pleomorphic nu-clei, with finely dispersed chromatin. There were increased mitotic and apoptotic activities in NECs compared to well-differentiated endocrine tumors. Neuroendocrine markers are immuno-reactive markers for diagnosis and indicate the neuroendocrine differentiation of tissue. CgA, Syn, NSE and CD56 as a biomarker panel for GEP-NENs can improve the sensitivity of diagnosis of GEP-NENs complementarily. CgA, Syn and CD56 are used as neuroendocrine markers for GEP-NENs. CgA is a neuroendocrine secretory protein, Syn is a synaptic vesicle glycoprotein present in neuroendocrine cells and CD56 is a neural cell adhesion molecule. In this study, CgA was positive in 66.7%, Syn positive in 77.1% cases, NSE in 60.4% cases and CD56 in 77.1% cases. In our study group, GEP-NENs diffusely expressed at least one neuroendocrine marker. In our study, MANEC had unequal adenoid structure and morphology consistent with small cell carcinoma with sheets and nests of polygonal cells displaying moderate nuclear pleomorphism and increased mitotic and apoptotic activity.

Ki-67 and mitotic activity are two markers used in the subclassification of GEP-NENs [1–2]. The GEP-NENs have been classified by the WHO (2010) in three grades (G1 to G3) based on mitotic activity and Ki-67/MIB-1 proliferation index [1]. These are G1: mitotic count < 2/10 HPF and/or Ki-67 proliferation index ≤ 2%. NEN G2 cells have a Ki-67 index of 3–20% and/or a mitotic count of 2–20 per 10 HPF. NET G1 and G2 cells are well-differentiated, the cells are round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei with uniformly dispersed coarse chromatin. However, GEP-NEC G3 cells are poorly differentiated and defined as NEC with mitotic count > 20/10 HPF and/or Ki-67 proliferation index > 20%. If the mitotic count or Ki-67 proliferation index points to different grades, a higher grade has to be given [9–12]. Some studies have shown discordance between mitot-ic count and Ki-67 index in some cases [8–10]. They have shown that the grade discordant tumors with a mitotic count of G1 and Ki-67 index of G2 behave worse than grade concordant tumors [9–10]. In our study, 33.3% of cases were G1, 12.5% were G2, 33.3% were NEC, and 20.8% were MANECs. Poorly differentiated tumors NEC and MANEC tend to have a higher Ki-67 index than do NET G1 and G2 tumor cells. Compared with the group with Ki-67 index less than twenty percent, the serum levels of CEA, NSE, and CA 19-9 were significantly higher in the group with Ki-67 index more than twenty percent in this study. In this study, the one-year and three-year survival rates were determined to be 72.2% and 61.1%, respectively. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC classification ($P < 0.05$), and metastases at diagnosis ($P < 0.05$) were associated with poorer prognosis. There was no significant correlation with sex, site, and age at diagnosis ($P > 0.05$).

As a heterogeneous disorder, GEP-NETs can be located in various anatomic sites in the abdomen, resulting in a wide range of clinical pictures and requiring the further inclusion of relevant clini-cians. The management of GEP-NETs requires the accumulation of knowledge and experience to establish a standardized approach.

GEP-NENs constitute a rare and heterogeneous group of tumors with varied biology and still constitute a diagnostic and therapeutic challenge for physicians of all specialties. These findings demonstrate that most GEP-NENs tumors are nonfunctional and present with nonspecific symp-toms. The most frequent primary site of the tumor was the rectum, and the age at diagnosis was 5th and 6th decades. Endoscopic biopsy is the main diagnostic and histological grading method for GEP-NEN. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC classification, and metastases at diagnosis were associated with poorer prognosis.

Conflict of interest

The authors confirm that this article has no conflict of interest.

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