CLINICOPATHOLOGICAL, HISTOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF NEUROENDOCRINE TUMORS OF GIT

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INTRODUCTION

Neuroendocrine tumors are relatively uncommon epithelial neoplasms with predominant neuroendocrine differentiation. They arise from most of the organs in the body. Some of the clinicopathological features of these neuroendocrine neoplasms are characteristic of the site of origin, while other characteristics are irrespective of their anatomic site.

Studies on neuroendocrine tumors have concentrated on tumors of specific organ systems such as gastrointestinal tract, pancreas and Lungs. Neuroendocrine tumors can arise anywhere in the gastrointestinal tract. But in the GIT, Neuroendocrine tumors are predominantly derived from the kulchitsky's cells or enterochromaffin cells. They present with different pathologic findings that corresponds to the site of origin and hormone secreting ability of these tumors.[46]

Gastrointestinal tract is the most common site to be affected.[16] The incidence of neuroendocrine tumors of GIT is around 67.5% among all neuroendocrine tumors.[53] These tumors develop throughout the gastrointestinal tract from oesophagus to anus although they are unusual in the oesophagus and anus.

Small intestine is the most common site of occurrence of neuroendocrine tumors in the git.[30] But most of the recent studies are against this concept. The pattern of neuroendocrine tumors of git has changed over the last few years. The prognosis of neuroendocrine tumors varies according to the grade of the tumor and is distinctly different from other types of malignancies that occur in GIT. The prognosis of neuroendocrine tumors is better than adenocarcinomas of the GIT. By histopathological evaluation and classification they are grouped into different prognostic categories according to their grade.

Histopathological diagnosis, tumor classification and identification of histogenesis of metastases of unknown or uncertain primary tumors are considered to be the most important responsibilities of practical histopathologists. At present, in addition to the traditional light microscopy, there is a list of other informative methods that support histopathologists in their work such as electron microscopy, histochemistry, immunohistochemistry and molecular methods.

In the past 20 years, immunohistochemistry has dramatically developed and has become a very powerful and simple tool in diagnostic histopathology. Many steps of immune-stain protocols were markedly simplified and a large number of diagnostic antibodies were introduced to

resolve many diagnostic problems and to increase the diagnostic accuracy.

As the size of the biopsies decrease, the role of immunohistochemical stains will become even more important in determining the origin and differentiation of gastrointestinal tract tumors. Immunohistochemical stains such as neuron specific enolase, chromogranin and synaptophysin are commonly used to identify neuroendocrine tumors.[37]

In this study we analyse the clinicopathological, histomorphological and immunohistochemical study of neuroendocrine tumors of gastrointestinal tract.

AIMS AND OBJECTIVES

1. To evaluate the clinical presentation of neuroendocrine tumors of gastro intestinal tract.

2. To evaluate their anatomical distribution in gastro intestinal tract.

3. To establish the histopathological type of neuroendocrine tumors of gastro intestinal tract.

4. To carry out immunohistochemical study with neuron specific enolase, synaptophysin and chromogranin A.

5. To correlate the histopathological type with immunohistochemical expression

REVIEW OF LITERATURE

The terminology for neuroendocrine tumors varies by anatomic site. Gastrointestinal neuroendocrine tumors have been variously described in the literature, due to their complexity and diversity. They were first described by T. Langhans and their team in 1867. In 1870, Heidenhain described neuroendocrine cells in the intestine based on their chromaffinity. In 1897 Nicholai Kulchitsky identified enterochromaffin like cells in the crypts of liberkuhn in the intestinal mucosa.

Gastrointestinal neuroendocrine tumors are traditionally known as carcinoids. The term “karzinoid”- “carcinoma like” was introduced in 1907 by Siegfried Oberndorfer. He found that some malignant intestinal tumors with distinct morphologic characteristics behaved less aggressively than adenocarcinomas in the same site.[37] Because of their slow growth they were considered to be “cancer-like” than truly cancerous. Due to the malignant potential of these neoplasms, the term gastrointestinal neuroendocrine tumors has been used now.

In 1906, Ciaccio described the neuroendocrine origin of carcinoid tumors and introduced the term enterochromaffin for these cells.[46] Huebschmann in 1910 found similarities between tumor cells and

kultschitzky cells in the crypts of Liberkuhn. Argentaffin positivity in these cells was demonstrated by Andre Gosset and Pierre Masson in 1914.[46] Subsequently endocrine tumors of the gastrointestinal tract and other sites were named carcinoids.

In 1929, Oberndorfer found the malignant behaviour of carcinoid tumors and modified his original description.[37] In 1931 Cassidy described that patients with these tumors present with cough, flushing, cyanosis and diarrhoea and the term 'carcinoid syndrome' was introduced. Rapport isolated and described serotonin in 1948. In 1953 the secretion of serotonin was confirmed by Lembeck in an ileal carcinoid. Carcinoid heart disease was identified in 1952 by Gunnar Biorck. Charles Moertel recognized the relationship between carcinoid tumors and fibrosis in 1961.[37]

The histochemical identification of argentaffin and argyrophil cells by Masson in 1914 and Grimelius in 1968, helped the pathologists to understand the nature of these tumors.[58] In 1969, Pearse described Amine Precursor Uptake and Decarboxylation (APUD) cells, postulating that they are derived from the neural crest and thus explained the origin of neuroendocrine cells. These cells have ability to uptake amine precursor substances and decarboxylate them to produce amines such as

serotonin and catecholamines. In 1987 Lechago, pointed out that not all endocrine cells [e.g., parathyroid] are capable of APUD, while some exocrine cell [e.g., paneth cells] are capable.[26]

One of the major contributions to the study of NETs came from J.C. Reubi of Bern in 1982 by identifying the cellular location of somatostatin receptors on neuroendocrine cells and tumors by using both radiolabelled somatostatin and immunohistochemical antibody techniques.[47]

In 1987, Lewin gave the concept of mixed tumors, and the term was restricted to those tumors in which atleast 30% of the bulk of the tumor was constituted by neuroendocrine cells. Lewin proposed to classify mixed tumors histomorphologically into three subtypes:

1. Ampicrine tumors
2. Collision tumors
3. Composite tumors.

In Ampicrine tumors the neuroendocrine and exocrine components are present within the same cell. In Collision tumor the two elements are

adjacent to each other in a side-by-side pattern. The two elements are intermingled in a Composite tumor.

In 1989, Somatostatin scintigraphy, the first imaging technique introduced in the diagnosis of Neuroendocrine tumors. This provides the therapeutic knowledge in treating somatotstin expressing lesions.

INCIDENCE and PREVALENCE

Neuroendocrine tumors are uncommon malignancies; bronchopulmonary and gastroenteropancreatic neuroendocrine tumors together accounts for only 0.5 to 1 percentage of all malignancies.

According to recent studies the incidence of neuroendocrine tumors is 2.5-5 per one lakh population.[41] Oyvind Hauso has found that the incidence of neuroendocrine tumors is on the rise.[15] The current prevalence is 35 per one lakh population.[41]

The primary carcinoid tumor of the stomach, lungs, appendix and caecum is more likely to occur in females. Males are more likely to have a primary neuroendocrine tumor in thymus, pancreas, duodenum, jejunum, ileum and rectum.[9] But most of the studies found a higher

incidence of Neuroendocrine tumors with in the gastrointestinal system in men than in women.

SITE

The majority of the neuroendocrine tumors occur in the GIT (67.5%) and the bronchopulmonary system (25.3%). Within the GIT, NETs occur in the small intestine (41.8%), rectum (27.4%) and stomach (8.7%). Less than 1% of the NETs occur in the pancreas.[9]

RISK FACTORS

Studies from United States found a higher incidence of carcinoid tumors in African American race when compared to Caucasians. Two studies found the association between tobacco smoking and small bowel carcinoid. But it is still in controversy because large studies did not find any correlation. Family history of MEN I and neurofibromatosis also have a risk for the development of carcinoids.

CLINICAL FEATURES

Most cases are asymptomatic and found incidentally. Symptoms can be due to the production of biologically active substances by tumor

cells. In non functioning tumors the symptoms may be due to the local mass effect or mesenteric fibrois. The common presenting symptoms in neuroendocrine tumors of GIT are as follows:

Symptoms

- Abdominal pain
- Vomiting
- Diarrohea (irrespective of flushing episodes)
- Intestinal obstruction
- Weight loss
- Bleeding per rectum

CARCINOID SYNDROME

A constellation of symptoms occurs in less than 10% of neuroendocrine tumors due to excessive levels of hormones like serotonin, substance P known as carcinoid syndrome.

The features of carcinoid syndrome are as follows:

- Cutaneous Flushing
- Abdominal cramping
- Asthma or wheezing
- Diarrhoea
- Palpitations
- Carcinoid heart disease and congestive cardiac failure
- Peripheral edema

Other manifestations include telangiectasia, pellagra-like skin lesions.

Carcinoid crisis occurs whenever there is a release of large amount of hormones in to the blood circulation or hypersecretion from tumors by trigger factors. Carcinoid crisis presents with, increased heart rate, profound flushing, unstable blood pressure and bronchospasm.

It can be triggered by factors such as food, alcohol, emotional events, defecation, embolization therapy, anaesthetic agents, surgery, radiofrequency ablation or chemotherapy. [9,46]

DIAGNOSIS

Neuroendocrine tumors of GIT usually present with obscure clinical features and require various investigations to establish the final diagnosis. The diagnosis is based on clinical features, biochemical analysis, imaging, and confirmation with histopathology.

BIOCHEMICAL ANALYSIS

Blood investigation

Chromogranin A, Serotonin, Gastrin and Histamine.

Urine analysis

24 - Hour urinary excretion of 5-hydroxy indoleacetic acid (5-HIAA). More than 6 mg / 24 hours - suggestive of carcinoid tumor. Normal urinary excretion ranges from 2-8 mg / 24 hours.

ULTRASOUND

Ultrasound scans of the abdomen

ENDOSCOPY

Upper esophagogastroduodenoscopy

Colonoscopy

Endoscopic ultrasound

IMAGING

CT, MRI,

PET (18F dopa PET)

SSRS – Somatostatin receptor analogue scan

^{111}In-Labeled somatostatin analogue (octreotide) scan

MIBG -Radiolabeled Meta-iodobenzylguanidine (^{123}I-MIBG)

Gastrointestinal endoscopy and advanced imaging techniques (CT & MRI) has now replaced the old diagnostic methods such as barium x-ray analysis and electroclysis. These imaging techniques are very much helpful in the diagnosis of metastatic lesions.

HISTOPATHOLOGY

Biopsy - Histopathology is the gold standard in the diagnosis of neuroendocrine tumors of GIT.

CLASSIFICATION OF NEUROENDOCRINE TUMORS

Neuroendocrine tumors are classified based on the site of origin, histomorphology and functional characteristics.

- Functional versus non functional
- Classification based on site of origin
- Histologic classification by WHO
- Classification by tumor stage: TNM
    - American joint committee for cancer (AJCC)
    - The European neuroendocrine tumor society (ENETS)
- Molecular classification

    MEN1 &2, Tuberous sclerosis,Von hippel lindau disease

FUNCTIONAL VERSUS NON-FUNCTIONAL

Neuroendocrine tumors can be classified as functional and non-functional based on the hormonal secretion. Functional neuroendocrine tumors are associated with symptoms that can be attributed to the secretion of specific peptides or hormones. Nonfunctional neuroendocrine tumors can also cause non specific symptoms related to increasing mass (pain, bleeding and obstruction) or metastasis (weight loss). Some neuroendocrine tumors can remain asymptomatic indefinitely.[21]

BASED ON SITE OF ORIGIN

Initially classification based on embryogenesis was putforth in 1963 by William and Sandler. They classified these tumors into foregut carcinoids, midgut carcinoids and hindgut carcinoids.

- Foregut Neuroendocrine tumors

  (Stomach, first part of duodenum, lungs)

- Midgut Neuroendocrine tumors

  (Appendix, right side of colon, jejunum, second part of duodenum) [13]

- Hindgut Neuroendocrine tumors

  (Rectum, sigmoid colon, transverse colon) [23,38]

Pancreatic endocrine tumors[34]

- Gastrinoma
- Glucagonoma
- Insulinoma
- Pancreatic polypeptideoma
- Somatostatinoma
- VIPoma

Additional sites

- Ovary
- Paraganglia
- Adrenal medulla

FAMILIAL SYNDROMES ASSOCIATED WITH NETs [19]

- MEN I & II
- Von Hippel Lindau disease
- Tuberous sclerosis
- Neurofibromatosis type I
- Carney complex

Classification based on histology

The classification of carcinoid tumors based on histological features was introduced by Jun Soga and Kenji Tazawa in 1971. They divided the carcinoid tumors according to their predominant growth pattern into insular, trabecular, glandular, mixed and undifferentiated. Insular pattern seen mostly in "midgut" carcinoids, while the others "foregut" and "hindgut" show a trabecular pattern.

The first WHO classification of neuroendocrine tumors was published in the year 1980. In 2000, the term 'carcinoid' was removed as it did not mean the real nature of the tumor which created confusion and provoked debate between the pathologists and the clinicians. So the term (neuro) endocrine tumor was introduced.

In 2000 classification, the neuroendocrine tumors were classified based on the histomorphology. In 2006, grading system was incorporated into the WHO classification proposed by ENETS which is based on proliferative rate of tumor cells and the recommended current classification is WHO 2010.

WHO 1980 CLASSIFICATION

I. CARCINOID

II. MUCOCARCINOID

III. MIXED FORMS CARCINOIDADENOCARCINOMA

IV. PSEUDOTUMOR LESIONS

WHO 2000 CLASSIFICATION

The World Health Organization (WHO) 2000 classification[6,41] of neuroendocrine tumors is based on behavioural characters, size of the tumor, depth of invasion and angioinvasion.

1. WELL DIFFERENTIATED ENDOCRINE TUMOR

    A) Benign behaviour

    B) Uncertain behaviour

2. WELL DIFFERENTIATED ENDOCRINE CARCINOMA

3. POORLY DIFFERENTIATED ENDOCRINE CARCINOMA

4. MIXED EXOCRINE-ENDOCRINE CARCINOMAS

WHO 2000 CLASSIFICATION

Character	**Well differentiated NET**	**Well differentiated NEC**	**Poorly differentiated NEC**
Biological behavior	Benign/low malignancy	Low malignancy	High malignancy
Metastasis	-	±	+
Ki-67 index(%)	<2	>2	>20
Histological differentiation	Good	Good	Poor
Infiltration/angioinvasion	Not present	Present	Present
Tumor size(cm)	≤2	>2	Any size

WHO 2010 classification

* NEUROENDOCRINE TUMOR Grade1(NET G1)

* NEUROENDOCRINE TUMOR Grade2 (NET G2)

* NEUROENDOCRINE CARCINOMA Grade3(NEC)

    - LARGE CELL NEC

    - SMALL CELL NEC

* MIXED ADENONEUROENDOCRINE CARCINOMA (MANEC)

Well differentiated NETs can be classified as either grade1 or grade 2 depending on cell proliferation and histology. Well differentiated grade1 and grade 2 NETs have traditionally been reported as carcinoids, regardless of grade or site of origin. According to WHO 2010 guidelines the term “carcinoid” applies to NET G1 only. Neuroendocrine carcinomas (NET G3) have a highly aggressive course with rapid dissemination and resistance to therapeutic interventions.

Comparison of WHO 2000 with WHO 2010 classification

WHO 2000	WHO 2010	HISTOLOGICAL FEATURES
Well differentiated endocrine tumor	NET G1	Well differentiated, mild to moderate nuclear atypia, corresponds to ENETS G1 by Ki67 labelling index and mitotic count
Well differentiated endocrine carcinoma	NET G2	Well differentiated, mild to moderate nuclear atypia, corresponds to ENETS G2 by Ki67 labelling index and mitotic count
Poorly differentiated endocrine carcinoma	NET G3	Poorly differentiated, marked nuclear pleomorphism, necrosis, corresponds to ENETS G3 by Ki67 labelling index and mitotic count
Mixed Endocrine – exocrine carcinoma	Mixed adeno-neuroendocrine carcinomas (MANEC)	Malignant tumors with mixed glandular and neuroendocrine characteristics, with atleast 30% of each component

THE EUROPEAN NEUROENDOCRINE TUMOR SOCIETY (ENETS) GRADING SYSTEM.

European Neuroendocrine Tumor Society (ENETS) proposed a histologic grading system based on mitotic rate and Ki-67 labelling index. The grading system is given below.[48,49]

Grade	Mitotic count (10 HPF) at 40X	Ki-67 % labelling index
1	< 2	Upto 2%
2	2-20	3-20%
3	>20	> 20%

* 10hpf (high power fields) is equal to 2 mm^2, at least 40 fields at 40x magnification should be evaluated in areas of highest mitotic density.

* Ki-67 index is the percentage of 2,000 tumor cells in areas of highest nuclear labelling.

* The grade 2 category identifies and recognises an intermediate group of NETs that shows a greater degree of pleomorphism, mitotic rate and Ki-67 labelling index than the grade 1 category.

WHO 2010 GRADING SYSTEM CRITERIA

Some of the biological behavior exhibited by neuroendocrine neoplasms is highly correlated with neoplasm grade. Placing a given tumor into one of categories depends on well-defined histological features, size, mitotic counts, Ki-67 labelling index, lymphovascular invasion, and invasion of adjacent organs, presence of metastases and whether they produce hormones.

* Grade 1 NETs are slow growing tumors
* Grade 2 NETs have a less predictable & moderately aggressive
* Grade 3 Neuroendocrine carcinomas can be highly aggressive

GRADE	CRITERIA
Low grade (G1)	Cytologically bland, mitotic count <2 / 10 HPFs and/or ≤ 2% Ki67 index
Intermediate (G2)	Cytologically bland, mitotic count 2-20 / 10 HPFs and/or 3%-20% Ki67 index
High(G3)	Mitotic count >20 / 10 HPFs and/or >20% Ki67 index
MANEC	Tumor has at least 30% of Adenocarcinoma or NEC

NEUROENDOCRINE TUMOR G1 (NET G1)

They can be divided into five histological patterns of growth

* Insular
* Glandular
* Trabecular
* Undifferentiated
* Mixed tumors

Their nuclei are regular; normochromatic with fairly uniform nuclei, salt-and-pepper chromatin, finely granular cytoplasm, scant mitoses and necrosis is absent and florid vascularisation.

NEUROENDOCRINE TUMOR G2 (NET G2)

NET G2 includes tumors that are more aggressive both histologically and clinically than well differentiated neuroendocrine tumors but are distinguished from poorly differentiated neuroendocrine carcinomas. It encompasses many tumors that were previously described by a variety of terms including “atypical” carcinoids, “malignant tumorlets” etc.[60]

NEUROENDOCRINE CARCINOMA (NET G3)

NET G3 includes tumors that are poorly differentiated with poor histological differentiation, mitoses >20 per 10 HPF, angioinvasion.[25]

MIXED ADENONEUROENDOCRINE CARCINOMA (MANEC)

MANECs have a carcinoma phenotype that is recognizable as both adenocarcinoma and neuroendocrine carcinoma. Each component should exceed at least 30% of all neoplastic cells. Both components should be graded. The identification in adenocarcinomas of scattered neuroendocrine cells (<30%) does not does not qualify under MANECs.[25]

TNM STAGING

TNM staging classification of the neuroendocrine tumors was initially proposed by European Neuroendocrine tumor society (ENETS) in 2006. In 2009, the American Joint Committee on Cancer has published a seventh edition of TNM staging manual that includes gastrointestinal and pancreatic carcinoids previously no such TNM staging for neuroendocrine tumors. TNM staging system has some prognostic value by giving information regarding the extent of local invasion, involvement of nodes and distant metastasis of the tumors. [See annexure for TNM staging system for neuroendocrine tumors of GIT]

GROSS:

Neuroendocrine tumors are small, yellow or tan masses, located in the submucosa or intramurally. They can be very firm due to an accompanying intense desmoplastic reaction. The overlying mucosa may be either intact or ulcerated. Some tumors invade deeply to involve the mesentery.

HISTOPATHOLOGY

NETs are an example of "small blue cell tumors," showing uniform cells which have round to oval nucleus with stippled chromatin and scant, pink granular cytoplasm. The cells may be arranged in islands, glands or sheets. High power examination shows bland histology. There is usually minimal pleomorphism but less commonly there can be anaplasia, mitotic activity, and necrosis. Histological pattern of these NETs were well explained above in the tumor grading.

ELECTRON MICROSCOPY

Electron microscopy reveals the neurosecretory or dense core granules of the neuroendocrine cells.[28]

IMMUNOHISTOCHEMICAL MARKERS

In recent years, Immunohistochemical analysis has been widely used in the diagnosis of neuroendocrine tumors of GIT. The ability to identify the cells of neuroendocrine differntitaion and the cells of hormonal secretion by immunohistohistochemical staining is proven helpful in the study of neuroendocrine tumors of GIT.

The useful neuroendocrine markers are Chromogranin A (CgA), Synaptophysin (P38), Neuron specific enolase (NSE, gamma-gamma dimer) and Protein Gene Product (PGP) 9.5. Newer markers introduced in the diagnosis of neuroendocrine cells are Hsp 70, CDX2 and NSP-55.

CHROMOGRANIN

Chromogranins and secretogranins are the major constituents of neuroendocrine secretory granules. Chromogranin & secretogranin family includes chromogranin A, chromogranin B, and Chromogranin C (Secretogranin II). Chromogranin proteins are distributed in the Neuroendocrine cells throughtout the body and the functions of these proteins are unknown. The chromogranin A, was the first to be discovered in the year 1965. Later it was purified from bovine adrenal medulla in 1967.[50] Chromogranin A is a highly acidic protein with a

molecular weight of 75000. It is an excellent marker for carcinoid tumors, pheochromocytomas, paragangliomas and other neuroendocrine tumors. In 1985, chromogranin B with a molecular weight of 100000 was identified in bovine adrenal medulla and was designated chromogranin B or secretogranin I. The predominant component of human chromaffin granules is chromogranin B.[50]

At ultra structural level chromogranin is present in dense core secretory granules the intensity of the immunostain depends on the number of neurosecretory granules in the cytoplasm of the cells that are examined. Neuroenocrine cells and tumors with numerous well developed secretory granules show intense positivity while paucigranular cells exhibit weak positivity. In paucigranular cels antibodies to other markers (eg. synaptophysin) may be positive.

Positive Control: Pancreas or adrenal gland

Cellular Localization: Finely granular positivity in cytoplasm

Normal Tissue: Pancreas

Abnormal tissue: Pheochromocytoma

SYNAPTOPHYSIN

Synaptophysin was first described and named by Wiedenmann. Synaptophysinn is encoded by the SYP gene. The other name of synaptophysin is synaptic vesicle protein p38. SYP gene is located on the short arm of X chromosome (Xp11.23-p11.22). It lies on the Crick (minus) strand is 12,406 bases in length. The encoded protein has 313 amino acids. The molecular weight of synaptophysin is 33.845 kDa.

It is a transmembrane calcium-binding glycoprotein present in the presynaptic vesicles with four transmembrane domains weighing 38kDa. It is present in neuroendocrine cells and all neurons in the brain and spinal cord that participate in synaptic transmission. Neuronal cells show a punctate pattern of staining corresponding to synaptic regions, while neuroendocrine cells show diffuse cytoplasmic staining pattern. Its ubiquity at the synapse has led to the use of synaptophysin immunostaining for quantification of synapses.[5]

At ultrastructural level synaptophysin is present in microvesicles. Cells with sparse granules that are chromaffin negative are positive for synaptophysin. Synaptophysin represents a more specific marker of neural structure than NSE.[20] The exact function of this protein is not known. It interacts with the synaptic vesicle protein synaptobrevin.

By immunohistochemical staining, it can be demonstrated in a variety of neural and neuroendocrine tissues, including pancreatic islets and cells of the adrenal medulla. Synaptophysin can be used to identify tumors have a origin from neuroendocrine cells such as neuroblastoma, phaeochromocytoma, carcinoid, and medullary thyroid carcinoma and others. For diagnostic purposes it is frequently used in combination with chromogranin A.

Positive Control: Pancreas, colon

Cellular Localization: Cytoplasmic positivity

Normal Tissue: Pancreas, colon

AbnormalTissue: Pheochromocytoma

NEURON SPECIFIC ENOLASE

Neuron specific enolase (NSE) is a glycolytic enzyme which catalyzes the reaction pathway between 2-phospho-glycerate and phosphophenol pyruvate. Enolases are homo or heterodimers composed of three subunits: alpha (α), beta (β) and gamma (γ). Antibodies to the gamma subunit are most commonly used. The gamma subunits are primarily expressed in neurons, normal and neoplastic neuroendocrine

cells. But they are also expressed in megakaryocytes, T- lymphocytes, striated and smooth muscle cells. NSE has a low specificity to neuroendocrine tumors. It is commonly used as a screening marker and the final diagnosis must be supported by other more specific markers.[2]

Cellular Localization: Cytoplasmic positivity

Normal Tissue: Pancreas, nerve

Positive Control: Pancreas or colon

Abnormal Tissue: Islet cell tumor, medullary and clear cell carcinomas

Chromogranin, synaptophysin and neuron specific enolase are the most commonly used neuroendocrine markers.

CHECKLIST FOR THE REPORTING OF NEUROENDOCRINE TUMORS OF GIT:

1. Location of the tumor.
2. Multiplicity – if any synchronous multiple tumors found in the specimen
3. Size

4. Well differentiated or poorly differentiated

5. Extent of local invasion and / or surgical margins

6. Lymphovascular space invasion - Present or absent

7. Perineural invasion - Present or absent

8. Proliferative rate using - mitotic count or Ki-67 labelling index

9. Lymph node status

10. Distant metastasis - Present or absent

11. Associated diseases e.g. chronic atrophic gastritis, inflammatory bowel disease, etc.

12. Somatostatin receptor status where applicable.

The summary of the pathology report should have tumor grade based on WHO 2010 classification (NET G1, NETG2, NEC and MANEC) and the TNM tumor stage for the specific site.

DIFFERENTIAL DIAGNOSIS

1. **HIGH- GRADE LYMPHOMA:** Sheets of pleomorphic & mitotically active blast cells, areas of necrosis. IHC: CD 45, B/T cell markers.

2. **EPITHELIOID GIST:** Sheets or nests of cells with eosinophilic to clear cytoplasm, round to ovoid nuclei, finely dispersed chromatin and prominent nucleoli. IHC: CD117, CD34.

3. **NEUROENDOCRINE CELL HYPERPLASIA**: Non-neoplastic proliferation of neuroendocrine cells. >5 coalescing nodules and each nodule with >5 endocrine cells in glands/crypts that do not exceed the diameter of gastric glands.

IMPORTANT POINTS ABOUT NEUROENDOCRINE TUMORS

1. Neuroendocrine tumors can arise most of the organs in the body, but shares certain basic characters irrespective of the site of origin.

2. Differentiation refers to the extent of resemblance to the normal cellular counterpart.

3. Grade refers to the degree of biologic aggressiveness which is more related with differentiation.

4. Stage refers to the extent of spread of the tumor.

5. A number of different systems exist to classify, grade, and stage the NETs.

6. Although the criteria differ among systems, the underlying basic data are similar.

7. The proliferative rate in the aspect of mitotic count or Ki-67 labelling index is a critical factor.

8. The local invasion into the organ of origin and involvement of regional lymph nodes or distant sites are critical factors.

9. Basic information should be included in the pathology reports, including grade and stage along with reference to the specific systems being used to define these parameters.[21]

IMMUNOHISTOCHEMISTRY

Immunological methods in diagnosis were first explained by Coons and Jones (Coons et al, 1940)[7] by using immunofluorescence technique in bacteria. Immunohistochemistry is based on the selective binding of specific immunologic reagents to specific antigenic determinants on a cell.

ANTIGEN

It is any foreign material that can enter the body and trigger a mechanism of immune response, which results in the production of antibodies.

ANTIBODY

It is a substance produced in response to an antigenic stimulus.

Immunohistochemistry is used to determine the expression of a particular antigen and its microanatomic location in a tissue. IHC uses antibodies by which antigenic differences between the cells can be identified. The lineage of cell population can be identified based on these differences and identifies biologically distinct population of cells within the same lineage.

Immunohistochemistry was first started in 1940 by Coons for frozen sections. Pierce in 1966, modified it and used for paraffin sections. Shi in 1991 introduced antigen retrieval technique. In antigen retrieval technique paraffin processed sections are heated at high temperatures before IHC staining. The use of antibody in immunohistochemistry depends on the sensitivity and specificity of antigen-antibody reaction.

Hybridoma technique provides limitless source of highly specific antibodies.

Advantages of immunohistochemical methods:

* Can be done on tiny biopsy specimens
* Fresh or frozen tissue is not required
* It can be done on routinely processed tissue sections
* Semi quantification can be done against cells that are negative for hormone receptor.
* Provides excellent morphological details and tissue localization.

* Tumor cells expressing hormones can be visualised on the microscope.

* Expenses are less.

* Provides us with a permanent preparation.

BLOCKING NON-SPECIFIC BACKGROUND STAINING:

Background staining occurs either due to non specific binding or presence of endogenous enzymes. Non-specific binding with polyclonal primary antibody can be minimised by pre-incubating sections with serum from the same species on optimal working dilution.

Endogenous enzymes such as peroxidase seen in normal and neoplastic tissues are abolished by peroxidase blocking or by using alternate systems such as immunogold technique. Methods suggested to overcome endogenous activity include incubation in methanol containing 0.5% hydrogen peroxide for ten minutes at room temperature.

DETECTION SYSTEMS:

Antibodies are labelled or flagged with fluorescent substances, heavy metals or enzymes to permit visualisation of the antigen. Enzymes are the most commonly used labels in immunohistochemistry . Incubation

with a chromogen using a standard histochemical method produces a stable coloured end product suitable for light microscopy.

METHODS:

DIRECT LABELLING METHOD:

Antibody is attached with a label by chemical means and directly applied to tissue sections. The advantage of this method is that they are simple to use. The main disadvantage is that the sensitivity is low in this method.

INDIRECT LABELLING METHOD:

Enzymes are labelled with a secondary antibody, which is produced against primary antibody. This technique is more sensitive.

AVIDIN BIOTIN CONJUGATE METHOD:

In this technique primary antibody is added followed by biotinylated secondary antibody and later by preformed complexes of Avidin and Biotin horse radish peroxidase conjugate. This method is more specific.

BIOTIN STREPTAVIDIN METHOD:

In this method instead of avidin biotin, Modified avidin biotin with streptavidin being used. The advantage of this method is that it produces less non specific background staining.

IMMUNOGOLD WITH SILVER ENHANCEMENT:

It can be used in both direct and indirect methods. It has found a wide role in ultrastructural immuno location. The gold particles are enhanced by addition of several layers of metallic sliver and then used. This technique may represent the most sensitive and effective light microscopy immunohistochemical method currently available.

IMMMUNO HISTOCHEMISTRY PROCESS

The tissue for IHC has to undergo fixation, dehydration and paraffin embedding as in routine H & E sections.

FIXATION

This is a critical step as preservation of morphology is essential for interpretation of IHC. The ideal fixative is 10% buffered formalin. According to sample dimensions, 10-24 hours fixation time was

followed. The disadvantage was that antigens were masked during fixation. It can be overcome by antigen retrieval technique.

According to immunohistochemical staining protocol, biopsies fixed for less than 6 hours or longer than 72 hours, or sample where fixation delayed for more than 1 hour do not give proper results.

ANTIGEN RETRIEVAL

This procedure involves unmasking of the antigens. The following technique can be used.

1. Proteolytic Enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

Care should be taken not to allow the section to dry after heating, as this destroys antigenicity. The nuclear details are not clear in poorly fixed tissues. Fatty tissues tend to detach from slides while heating.

CONTROLS

Use of control tissue is essential in immunohistochemistry. Use of internal control protects against the effects of poor fixation.

MATERIALS AND METHODS

This study was conducted in our Department of Pathology at Kilpauk Medical College, Chennai. After approval from ethical committee of our institution, a total of 53 cases of neuroendocrine tumors of GIT were included in the study design. All the specimens were received between September 2008 and September 2012 from the Department of Surgery and the Department of digestive health diseases, kilpauk medical college, Chennai.

In each patient the clinical data, including gender, age at the time of diagnosis, clinical presentation, anatomic site and operative findings (if present) were obtained from the medical records.

Among the 53 specimens, 45 were biopsies and 8 were resection specimens. Histopathological study was done in all the specimens as per standard guidelines. Tumor grading was assigned using the mitotic count criteria, according to the WHO 2010 classification. Immunohistochemical analysis was done in 40 cases using antibodies against Neuron specific enolase, Synaptophysin and Chromogranin.

HISTOPATHOLOGICAL STUDY

All the specimens received were fixed in 10% neutral formalin for 18 - 24 hours. Detailed gross examination of the specimen was done. Representative samples were taken. The tissues were processed in various grades of alcohol and xylol using automated histokinette.

The tissue was processed for routine histopathological examination as follows:

PROCESSING OF HISTOLOGICAL SLIDES

1. Sections of 4-5 μm thickness were cut from processed paraffin embedded blocks and then gently lowered on the surface of water bath at 45ºC.

2. The sections were taken on alcohol cleaned glass slides smeared with a thin film of egg albumin.

3. The slides with sections were warmed on hot plate at 58ºC for 1 hour, cooled and stored in a box for staining.

4. Removal of wax was done with xylene. Slides were kept in xylene for 2 minutes and 2 such changes were done.

5. Xylene was removed with absolute alcohol. The slides were then kept in absolute alcohol for 2 minutes and two such changes was made.

6. The sections were treated with descending grades of alcohol with 90% alcohol for 1 minute and in 70% alcohol for 1 minute.

7. Finally sections were brought into deionised water. The sections so obtained were processed for H & E staining.

HAEMATOXYLIN AND EOSIN STAINING PROCEDURE

1. Sections are stained in a solution of Harris hematoxylin for 5-15 minutes.

2. Washed thoroughly in running water for 15-30 seconds.

3. Sections were decolorized with 1% acid alcohol solution for 10-20 seconds.

4. Sections are again washed with tap water.

5. Keep in warm water for 5 minutes.

6. Counter stained with 1% aqueous Eosin for 1-15 minutes.

7. Washed rapidly in water to remove excessive amounts of eosin.

8. Dehydrate by several changes of increasing grades of alcohol.

9. Cleared in xylene and mounted with Dextrin 80 di-butyl phthalate xylene(DPX) mountant.

Results

Cytoplasm-Pink

Nucleus-Blue

Immunohistochemistry was done in 40 cases. Suitable blocks were chosen for IHC. The immunohistochemical stains used were Neuron specific enolase, Synaptophysin and Chromogranin.

Sections for Immunohistochemistry were also cut in microtome using disposable blades. Slides coated with chrome alum were used. Sections were subjected to antigen retrieval using pressure cooker technique using citrate retrieval solution (pH 6) and then treated by Horse Radish Peroxidase (HRP) polymer techniques.

IMMUNOHISTOCHEMICAL STAINS

The following Immunohistochemical antibodies were used from the Biogenex laboratories.

1. Neuron specific enolase, Mouse monoclonal (MIG-N3).
2. Chromogranin A, Mouse Monoclonal (LK2H10), IgG1, Kappa
3. Synaptophysin, Mouse Snp 88, IgG3, Kappa.

METHODOLOGY

Coated slides after antigen retrieval were taken through following stages.

1. Treatment with peroxidise block for inhibiting endogenous peroxidises in the tissue for 5 minutes.
2. Washed two times in TRIS buffer for 5 minutes.
3. Application of power block for blocking non-specific antigen- antibody reaction for 5 minutes.
4. Washed two times in TRIS buffer for 5 minutes.

5. Application of primary antibody for 60 minutes.

6. Washed two times in TRIS buffer for 5 minutes.

7. Application of secondary antibody with the tagged Horse Radish Peroxidase enzyme for 30 minutes.

8. Washed two times in TRIS buffer for 5 minutes.

9. Application of super enhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen - antibody reaction.

10. Washed two times in TRIS buffer for 5 minutes.

11. Application of DAB (Diamino benzidine) chromogen for 5 minutes - this is cleaved by enzyme to give the coloured product.

12. Washed in distilled water for 5 minutes.

13. Counterstaining of slides done with hematoxylin.

14. Air dried and mounted with DPX.

RESULTS

Neuron specific enolase:

Positive: Cytoplasmic-brown

Nucleus –blue.

Synaptophysin:

Positive: cytoplasmic , membranous or granular-brown

Nucleus: blue

Chromogranin:

Positive: Granular cytoplasmic – brown

Nucleus: blue

OBSERVATION AND RESULTS

TABLE - 1

INCIDENCE OF NEUROENDOCRINE TUMORS

Duration of study (4 years)	Total no of specimens	Total no of malignancies	Total GIT malignancies	NET of GIT
September 2008 To September2012	20828	1423	886	53

The Total number of specimens received during the period of 2008 (September) to 2012 (September) were 20828. Out of the 20828 specimens, 886 specimens were GIT malignancies. Among the 886 specimens 53 specimens were diagnosed as neuroendocrine tumors of the GIT.

In our study the incidence of neuroendocrine tumors of GIT over the period of four years was 5.98 %. The incidence of neuroendocrine tumors of the GIT among the total malignancies was 3.72%.

TABLE - 2

AGE DISTRIBUTION OF NEUROENDOCRINE TUMORS OF GIT

AGE GROUP (years)	CASES	
	NUMBER	PERCENTAGE (%)
21-30	3	5.7
31-40	11	20.8
41-50	14	26.4
51-60	15	28.3
61-70	9	17.0
71-80	1	1.9
TOTAL	53	

This table shows the incidence of neuroendocrine tumors of GIT in different age groups. In our study the youngest person affected was 25 years and the eldest one was 71 years old. The maximum number of cases [15/53, (28.3%)] reported was between 51and 60 years of age. About 74% of the cases were more than 40 years with the median age of 50 years.

TABLE - 3

GENDER DISTRIBUTION OF NEUROENDOCRINE TUMORS OF GIT

GENDER	NO OF CASES	PERCENTAGE (%)
Male	32	60.37
Female	21	39.62
TOTAL	53	

In our study, the occurrence of NET of GIT was more common in males when compared to females. Among 53 specimens, 32 specimens belonged to male patients and 21 specimens were from female patients.

In our study, the incidence of NET of GIT was higher in males with 60.37%. The observed male: female ratio is 1.5:1.

TABLE - 4

COMPARISION OF CLINICL FEATURES

SYMPTOMS	NO OF CASES	PERCENTAGE (%)
Loss of weight and appetite	21	39.6
Vomiting	9	16.9
Abdominal pain	28	52.8
Diarrhoea	2	3.7
Obstructive jaundice	5	9.4
Bleeding Per Rectum	4	7.5

In our study the most common presenting clinical feature was abdominal pain. 28 (52.8%) patients presented with abdominal pain followed by loss of weight and appetite in 21 (39.6%) patients. Diarrhoea was a rare presentation seen only in 2 (3.7%) patients.

TABLE – 5

CARCINOID SYNDROME

TOTAL NO OF CASES	CARCINOID SYNDROME	PERCENTAGE (%)
53	02	3.8

Out of the 53 cases Carcinoid syndrome features were present only in 2 cases. In our study the incidence of Carcinoid syndrome is 3.8%.

TABLE - 6

COMPARISON OF TYPE OF SPECIMEN RECEIVED

SPECIMEN	NO OF SPECIMEN	PERCENTAGE (%)
Biopsy	45	85
Resection	8	15
TOTAL	53	

Among 53 samples of NET of GIT, 45 were biopsy specimens and 8 were resection specimens. In our study biopsy specimens constitute large proportion with 85% when compared to resection specimens which is only 15%.

TABLE - 7

SITE DISTRIBUTION OF NET OF GIT

SITE	NO OF CASES	PERCENTAGE (%)
Oesophagus	1	1.9
Stomach	24	45.3
Small Intestine	18	33.9
Appendix	1	1.9
Colon	5	9.4
Rectum	4	7.5
TOTAL	53	

Among 53 cases, 24 specimens from stomach, 18 from small intestine and the specimens from other sites colon, rectum, appendix, & oesophagus were 5, 4, 1&1 respectively. In our study the most common site involved was stomach constituting 45.3% followed by small intestine 33.9%. The least common were appendix and oesophagus with one case each. Colon and Rectum were intermediate with 9.4% and 7.5% respectively.

TABLE - 8

HISTOLOGICAL GRADING

DIAGNOSIS	NO OF CASES	PERCENTAGE (%)
Neuroendocrine tumor grade1 (NET G1)	22	41.5
Neuroendocrine tumor grade2 (NET G2)	9	17
Neuroendocrine carcinoma (NEC)	4	7.5
Mixed adenoneuroendocrine carcinoma (MANEC)	18	34
TOTAL	53	

Histological grading was done according to WHO 2010 classification to compare the histopathological pattern of NET of GIT. In our study the Neuroendocrine tumor Grade 1(NET G1) was found in 22 cases followed by Neuroendocrine tumor G2 (NET G2) in 9 cases and Neuroendocrine carcinomas (NEC) in 4 cases. Mixed

Adenoneuroendocrine carcinoma (MANEC) found in 18 (34%) cases irrespective of the tumor grade, can be either well differentiated (NET G1/ G2) or poorly differentiated (NEC) and these cases were not included in any of the tumor grade (G1to NEC) and dealt separately in this study.

In our study the most frequent histological grade was neuroendocrine tumor G1 with 41.5% and the least one was neuroendocrine carcinoma with 7.5%.

TABLE - 9

SITE WISE DISTRIBUTION OF NEUROENDOCRINE TUMORS

SITE	NET GI	NET G2	NEC	MANEC	TOTAL
OESOPHAGUS	1 (100%)	0 (0)%	0 (0)%	0 (0)%	1
STOMACH	8 (33.3%)	6 (25%)	1 (4.2%)	9 (37.5%)	24
SMALL INTESTINE	10 (55.6%)	2 (11.1%)	1 (5.6%)	5 (27.8%)	18
APPENDIX	1 (100%)	0 (0)%	0 (0)%	0 (0)%	1
COLON	0 (0)%	0 (0)%	2 (40%)	3 (60%)	5
RECTUM	2 (50%)	1 (25%)	0 (0)%	1 (25%)	4
TOTAL	22	9	4	18	53

The above table shows the anatomical site distribution of neuroendocrine tumors according to WHO 2010 grading. Large

proportion of cases reported from stomach & small intestine and least number of cases from appendix and oesophagus. In the stomach major histological differentiation was MNAEC with 37.5%, followed by NET G1with 33.3%, and NET G2 accounted for 25%. But in small intestine majority of cases were NET G1 with 55.6% followed by MANEC with 27.8%, and NET G2 accounted for 11.1%.

Among the five cases in colon, 2 were NEC and 3 were MANEC. Among that occurred in Rectum 2 were NET G1, 1 was NET G2, and 1 was MANEC. Histological differentiation found in appendix and stomach was NET G1.

TABLE - 10

METASTASIS

TOTAL NO OF CASES	LIVER METASTASIS	PERCENTAGE (%)
53	03	5.7%

Metastasis was seen in 3 cases. In all the cases the site of metastasis was the liver. The incidence of metastasis to liver was 5.7%.

TABLE-11

AGE	GENDER	PRIMARY SITE	GRADE
48	M	COLON	MANEC
50	M	RECTUM	MANEC
0	F	STOMACH	NET G2

The primary sites for the liver secondaries were colon, rectum and stomach. The histological diagnosis of the primary sites was found to be MANEC in colon & rectum and NET G2 in stomach. Among 3 cases, 2 patients were male and all of them were in the 4^{th} decade of age.

TABLE - 12

IMMUNOHISTOCHEMICAL EXPRESSION OF NET OF GIT

MARKERS	POSITIVE CASES	PERCENTAGE
NEURON SPECIFIC ENOLASE	38	95
SYNAPTOPHYSIN	35	87.5
CHROMOGRANIN	33	82.5
TOTAL NO OF CASES	40	

Immunohistochemical staining was studied in 40 cases out of 53. In which, neuron specific enolase was positive in 38 cases, synaptophysin was positive in 35cases and chromogranin was positive in 33 cases. When we compared the expression of immunohistochemical marker, higher incidence was found in neuron specific enolase with positive percentage of 95% followed by synaptophysin (87.5%) and chromogranin (82.5%).

TABLE - 13

EXPRESSION OF NSE WITH RELATION TO TUMOR GRADE

GRADE	POSITIVE	NEGATIVE	TOTAL
NET G1	19	0	19
NET G2	7	0	7
NEC	3	1	4
MANEC	9	1	10
TOTAL	38	2	40

[Chi-square value= 5.263 (df-3)

p value=0.154. The distribution is not significant (p >0.05)]

Both NET G1 and NET G2 expressed positivity for Neuron specific enolase in all cases. In NEC 3cases were positive and one was negative. In MANEC 9 cases were positive with one negative. In this study the correlation between the tumor grade and the expression of Neuron specific enolase was not statistically significant (p>0.05). Neuron specific enolase positivity was seen in most of the cases (38/40) irrespective of the tumor grade.

TABLE - 14

EXPRESSION OF SYNAPTOPHYSIN WITH RELATION TO TUMOR GRADE

GRADE	POSITIVE	NEGATIVE	TOTAL
NET G1	19	0	19
NET G2	6	1	7
NEC	2	2	4
MANEC	8	2	10
TOTAL	35	5	0

[Chi-square value = 8.392 (df-3)

p value = 0.039.* The distribution is significant ($p<0.05$)]

Out of the 40 cases positivity for synaptophysin was seen in all cases of NET G1. In NET G2, 6 cases were shown positive results and one was negative. In NEC, 2cases were positive and 2 were negative. In MANEC, 8 cases were positive with 2 negative results. The association

between tumor grade and the expression of synaptophysin was statistically significant ($p<0.05$) at 95% confidence interval.

In our study we found that expression of synaptophysin has significant correlation with tumor grade in most of the cases of NET of GIT.

TABLE - 15

EXPRESSION OF CHROMOGRANIN WITH RELATION TO TUMOR GRADE

GRADE	POSITIVE	NEGATIVE	TOTAL
NET G1	18	1	19
NET G2	6	1	7
NEC	2	2	4
MANEC	7	3	10
TOTAL	33	7	0

[Chi-square value = 6.029 (df-3)

p value = 0.110. The distribution is not significant ($p>0.05$)]

Chromogranin expressed positivity for 18 cases in NET G1, 6 cases in NET G2, 2 cases in NEC and 7 cases in MANEC from the total of 19, 7, 4, and 10 cases respectively. The expression of Chromogranin was statistically not significant ($p>0.05$).

The negative expression pattern of chromogranin was more when compared to other markers [7 cases with CgA, but only 5cases & 2 cases gave negativity with SYN and NSE respectively]. In our study the tumors expressing chromogranin was less. So it becomes a less sensitive marker in the diagnosis of NET of GIT even though it has some specificity.

DISCUSSION

Gastrointestinal Neuroendocrine tumors are rare malignant tumors. Due to improved diagnostic and therapeutic modalities, they have gained attention over the last few years. From all over the world, there is limited epidemiological data available for neuroendocrine tumors of GIT. In India, studies conducted on neuroendocrine tumors of GIT are less. Hence, this study was planned to determine the pattern of NET of GIT in our population.

After approval from our ethical committee, this descriptive study was conducted in our department of pathology at Kilpauk medical college & hospital. In this study, a total of 53 specimens received between September 2008 and September 2012 diagnosed as having neuroendocrine tumors of GIT were analysed for histopathological and immunohistochemical expression.

At present, it is estimated that the incidence of GEP-NETs is approximately 2.5 to 5.0 cases per 100,000 in the United States which indicates the low incidence of these tumors.[41] But the SEER database suggests that their prevalence has increased dramatically over the last three decades. In fact, it is believed that the incidence of these tumors is increasing globally. It is likely that this increase is due to an increase in

the actual number of cases and/or increased clinical and pathological experience with diagnosing this disease.[15]

In our study the incidence of Neuroendocrine tumors of the GIT constitute about 3.72% of total malignancies. Among the GIT malignancies they constitute about 5.98 % which is higher when compared to the studies conducted by Maroun et al[31] (constitute <2% of all GIT malignancies) and Niederle et al[40] (constitute 1.49% of the malignancies of digestive tract).

AGE

The median age at diagnosis of NET of GIT in the present study was 50 years with the age range between 25 years and 71 years. Most of the studies correlated with our study having average age at initial diagnosis between 50 and 60 years.

Amarapurkar et al1 have done a retrospective analysis of NET of GIT and Pancreas in 74 patients. In their study the mean age at diagnosis was 53.01±15.13 years. Estrozi and Bacchi9 found an average of 52.8 years in 773 cases of Gastroenteropancreatic Neuroendocrine tumors. Rothenstein et al[51] also showed the mean age of 56 years in their study of 193 patients with NET of the gastrointestinal tract which demonstrated

that 72% were NET/carcinoids. Neuroendocrine tumors were rare in paediatric age group.

SEX DISTRIBUTION

In our study males had higher incidence of NET of GIT with 60.37%. The observed male: female ratio is 1.5:1. Most of the studies correlated well with our study. The same male preponderance was observed by Yao et al.2008 (M: F=1.2:1) in USA and by Ito et al.2010 (M: F 2:1) in Japan and also by Niederle et al. 2010 (M: F=1.08:1) in Austria.[40] Rothenstein et al & Amarapurkar et al also showed that males were commonly affected with M: F ratio of 2:1. In contrast, Estrozi and Bacchi found higher number of neuroendocrine tumors in females.

SITE DISTRIBUTION OF NET OF GIT

Overall, the GIT represents the site of greatest NET incidence (64.3%), followed by the bronchopulmonary system (27.9%). In our study the most common site involved is Stomach constituting 5.3% followed by small intestine 33.9% and large intestine (Colon - 9.4%, Rectum - 7.5%). This is in contrast to previous studies (Modlin et al. 2003, Maggard et al. 2004, Helland et al. 2006, Borislav et al 2007 and Lombard - Bohas et al. 2009)[40] stating the small intestine as the most

frequent site. Over a decade the controversy is existing between the small intestine and the stomach as the common site of occurrence of NET. Initially, Kloppel et al. 2007 suspected stomach as the preferential site of NET of GIT. Now most of the studies revealed that the stomach as the commonest site of NET of GIT. Following Kloppel et al, Niederle et al (2010) also reported that stomach was the commonest site (22.8%) followed by appendix (21%). A study conducted by Amarapurkar et al (2010) in India with stomach (30.2%) being the common site followed by pancreas (23.3%). Estrozi and Bachi (2011) also found that stomach (24.5%) was the most common anatomic location followed by small intestine (20.5%).

Hodgson et al[17] in 2005 have shown statistically significant increae of about eight to nine fold increase in the incidence of gastric neuroendocrine tumors. Modlin et al[37] have shown significant increase in incidence of gastric neuroendocrine tumors from 2.4 to 8.7 %. A study from India by Hegde et al[16] has also shown rising incidence of gastric NETs as compared to the past.

An explanation for the increase may be the greater use of endoscopic diagnostic procedures and biopsies as a routine for all cases, even with small gastric lesions. The widespread use of proton pump

inhibitors and increase in endoscopic biopsies has been found to be the reasons for the increased incidence of neuroendocrine tumors of GIT.

The most common symptom presented in our study was abdominal pain and other symptoms were vomiting, loss of weight and appetite, diarrhoea, bleeding per rectum etc.

According to Niederle & Niederle (2011),[40] the most common symptom was abdominal pain. About 29.5% (71 of 241) of cases presented with abdominal pain in their study which correlates with our study.

Carcinoid syndrome is frequently discussed in relation to carcinoid tumors. Carcinoid syndrome found in 3 (4.1%) patients out of the 74 patients in a study conducted by Amarapurkar et al is very much comparable with our study were carcinoid syndrome found in 2 patients (3.8%). However, the complex of flushing, diarrhoea, abdominal pain, and occasional asthma or right-sided valvular problems is actually uncommon. Ito et al and Soga et al study also show that less than 10% (1.7%-8.4%) of neuroendocrine tumors exhibit some of these symptoms[18,54]. The conducted by Warrell et al. (2003)[59] Carcinoid syndrome occured in 10 % of the cases.

HISTOPATHOLOGICAL GRADING

In our study histological grading was done according to WHO 2010 classification published recently. The most frequent histological grade was found to be Neuroendocrine tumor G1 (NET G1) with 41.5% and the last one was Neuroendocrine carcinoma (NEC) with 7.5%.

Most of the studies show that the majority of neuroendocrine tumors of GIT belong to G1, which correlated with our study. Estrozi and Bacchi (2011) used WHO 2010 classification and the ENETS scheme in their study and found G1 (NETG1, with a mitotic count of <2 per 10 high power fields (HPF) and a Ki-67 index ≤2%) tumors are the commonest with 73.2%. Niederle & Niederle (2011) reclassified 77 cases of gastroenteropancreatic neuroendocrine tumors according to WHO 2010 classification and they also concluded that the majority of neuroendocrine tumors of GIT were G1 (59.7%) and G2 (31.2%) independent of their staging. In their study the NET G3 (NEC-Neuroendocrine carcinoma) are very rare and found in stomach, colon and rectum with 9.1% which is near comparable to our study with 7.5 % of NEC diagnosed in stomach, colon and small intestine. Borislav et al (2007) showed that out of the 38 cases, 29 were G1, 7 were G2, and 2 were G3 based on WHO 2000

classification which is nearly equal to NET G1, NET G2, and NEC of WHO 2010 classification.

The presence of a neuroendocrine component in gastrointestinal adenocarcinoma is often reported. Similarly, the presence of an exocrine component in NET of GIT, especially in high grade neuroendocrine carcinomas, has also been widely documented. There is a wide spectrum of such combinations of exocrine and neuroendocrine components ranging from adenomas or carcinomas with interspersed neuroendocrine cells at one end to classical neuroendocrine tumors with a focal exocrine component on the other end. In the 2000 WHO classification such neoplasms were defined as mixed exocrine-endocrine tumors when each component represents at least 30% of the lesion. In the most recent WHO 2010 classification of neoplasms of the gastrointestinal tract, such neoplasms are called “mixed adenoneuroendocrine carcinomas” (MANECs). Mixed Adenoneuroendocrine Carcinomas (MANECs) have a carcinoma phenotype that is recognizable as both adenocarcinoma and neuroendocrine carcinoma with each component exceeding at least 30% of all neoplastic cells & both components should be graded. The identification in adenocarcinomas of scattered neuroendocrine cells (<30%) does not qualify for MANEC.

In our study 34% (18/53) of cases showed Mixed Adenoneuroendocrine carcinoma (MANEC) which is irrespective of the tumor grade. These cases were not included in the any of the tumor grade even though they can be classified as well differentiated (NET G1/ G2) or poorly differentiated (NEC) at present study. Previous studies regarding MANEC were mostly case reports or just documentation. An update on MANEC by La Rosa et al (2012)[25] informs that approximately 100 documented cases have appeared in the literature. Most of these neoplasms have been reported in the oesophagus, stomach, ampullary region, large bowel and anorectal region. Most of the MANEC with NET component were commonly found in stomach with male preponderance, around the age of 53 years and with liver metastasis in a few cases, which correlates with our study. In our study MANEC was found mostly in stomach with higher incidence rate (37.5%) followed by the small intestine (27.8%). Most of the studies discussed above have not studied the MANEC or mixed exocrine-endocrine tumors. Now it is important to classify them because gastric MANEC shows a better prognosis than gastric NEC. The diagnosis of MANEC mostly incomplete because frequently only one component of the neoplasm is identified. Now it is important to classify them because gastric MANEC shows a better prognosis than gastric NEC.[24] So the discussions regarding MANEC

require further studies based on WHO 2010 classification of NETs of GIT.

IMMUNOHISTOCHEMISTRY

Various studies have published on immunohistochemical expression of NET of GIT from 1996 (Blumenfeld W et al)[4] to till date. Most of the studies confirmed that Neuron Specific Enolase (NSE), Synaptophysin and Chromogranin were useful markers in the diagnosis of NET of GIT.

When we analyse the recent studies, the positivity rate for NSE, SYN and CgA was 100%, 100% and 61.9% by Fen-Yau Li et al[10] (2010); 85.7%, 100% and 42.9% by Uchiyama et al[56] (2012); which is comparable with our results of 95%, 87.5% and 82.5% respectively. In our study we found that expression of Synaptophysin has significant correlation with tumor grade in most of the cases of NET of GIT. Even though Synaptophysin has significant correlation, we cannot use a single marker to confirm the diagnosis of neuroendocrine tumors. Due to the difference in the positive percentage, no single marker can be relied on exclusively and the use of adequate panel of markers is always advisable to confirm or exclude the final diagnosis.

The immunoreactivity with negative expression pattern was more with Chromogranin when compared to other markers [7 cases with CgA, but only 5 cases & 2 cases gave negativity with SYN and NSE respectively] in our study. And the number of cases showing negative expression was more with NEC and MANEC when compared to NET G1 & NET G2. According to Rindi et al[49] the Well Differentiated NETs tend to exhibit diffuse and intense expression of CgA and synaptophysin, whereas Poorly Differentiated neuroendocrine carcinomas show significantly reduced CgA expression while maintaining intense staining for synaptophysin. Chromogranin positivity generally correlates with the extent of granularity on electron microscopy. The intensity of the immunostain depends on the number of neurosecretory granules in the cytoplasm of the cells examined as in small cell carcinoma, which synthesizes actively chromogranin, but because of paucity of cytoplasm and scarcity of neurosecretory granules, shows usually very weak chromogranin stain. Chromogranin A may be negative in Somatostatin positive duodenal NET and Rectal NET.

Neuron-specific enolase is a cytoplasmic enzyme detected in tumors of neuroendocrine differentiation, but lacks specificity compared to CgA and synaptophysin. NSE usually used as a screening marker and the diagnosis must be supported by other more specific markers.

Immunohistochemical expression by neuroendocrine portion of the mixed adenoneuroendocrine carcinoma will be most useful in diagnosing the MANEC.

Immunohistochemical study with neuroendocrine markers may be very useful to confirm the nature of the tumor based on endoscopic tiny biopsy specimens in many cases where histological diagnosis becomes difficult. In distant metastasis, immunohistochemical study gives accurate definite diagnosis of origin and differentiation of primary neuroendocrine tumors. Thus immunohistochemistry has a definite role in the diagnosis of neuroendocrine tumors of GIT.

SUMMARY AND CONCLUSION

Neuroendocrine tumors of the GIT, known to be rare tumors, presents with increased incidence over the recent decades, most probably due to the increased awareness among the physicians and improved diagnostic techniques.

In this study we analysed the clinicopathological and immunohistochemical characteristics of 53 neuroendocrine tumors and the findings are summarized below.

1. Neuroendocrine tumors of the GIT constituted 3.72% of all malignancies. Among the GIT malignancies they constitute about 5.98 % of the malignancies.

2. The common age group affected was 51-60 years with the median age of 50 years.

3. Males were commonly affected, with male: female ratio of 1.5:1.

4. Stomach being the common site with 45.3% followed by small intestine with 33.9%.

5. Most common tumors diagnosed were neuroendocrine tumor G1 (41.5%) followed by NET G2 (17%). Mixed adenoneuroendocrine carcinomas presented with equal incidence with NET G1.

6. The positivity rate for neuron specific enolase, synaptophysin, and chromogranin was 97.5%, 87.5%, and 82.5% respectively. Synaptophysin had significant correlation with tumor grade. Even then a panel of markers should be used to prevent the error in diagnosis.

To conclude, neuroendocrine tumors of GIT are distinct neoplasms that differ clinically, histomorphologically and immunohistochemically from other tumors of GIT. So confirmation of histopathological diagnosis by immunohistochemistry is important to give a definitive diagnosis as the prognosis and treatment of these tumors differs from other tumors. Immunohistochemistry should be done with a panel of markers to avoid the error in diagnosis.

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Fig 1: Gastrectomy specimen showing proliferative growth measuring 5x4 cm in NET G1

Fig 2:Subtotal gastrectomy specimen with ulcerative growth measuring 8x7cm in NET G3

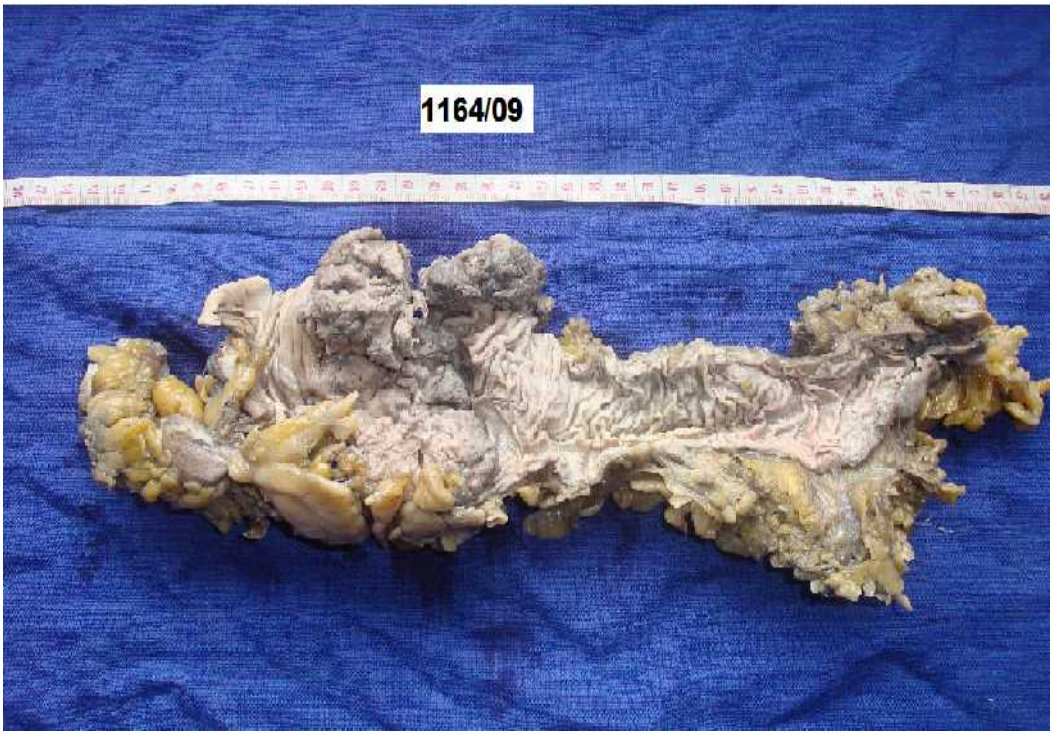


Fig3: Hemicolectomy specimen showing ulceroproliferative growth measuring 6x5 cm in MANEC

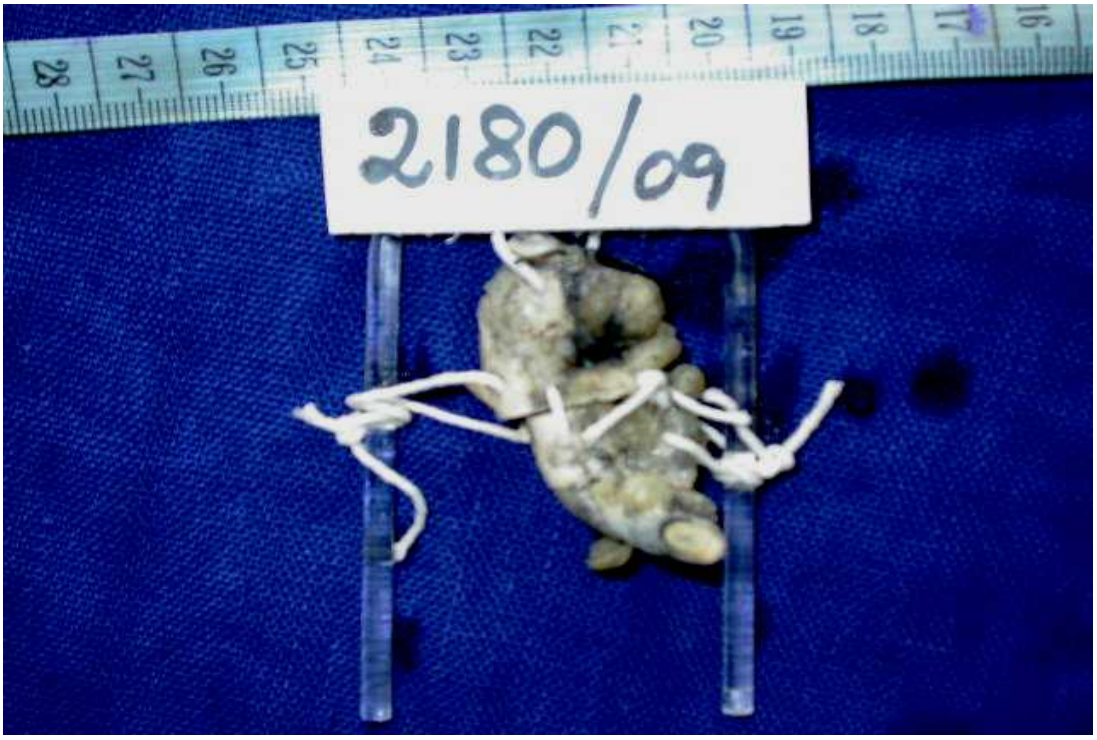


Fig 4: Appendicectomy specimen measuring 5cm in length. C/S tip shows a small circumscribed tan yellow mass measuring 0.5cm in NET G1

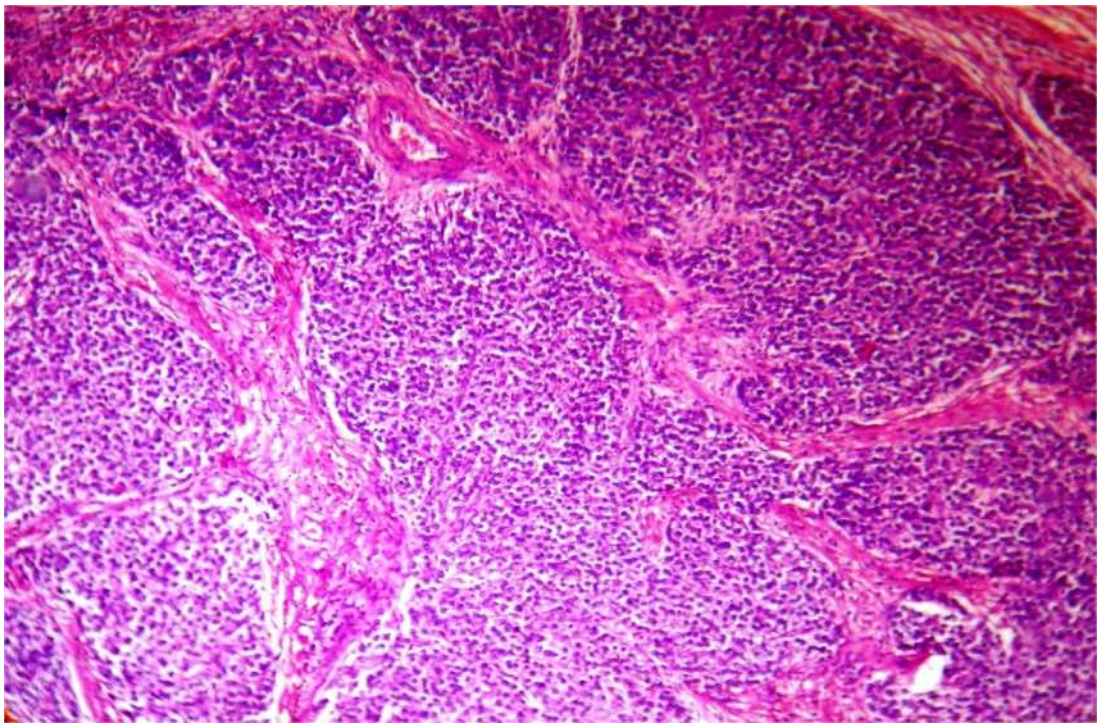


Fig 5: NET G1 arranged in nests and sheets. H&E10x view

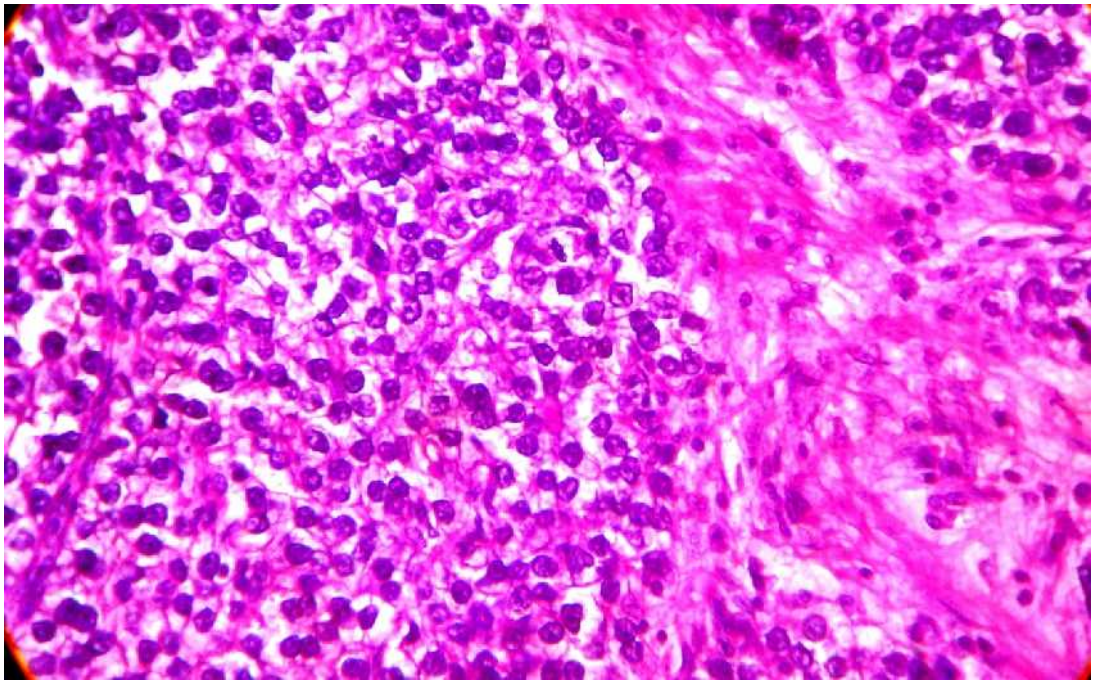


Fig 6:Cells with scant eosinophilic cytoplasm and regular nuclei, salt and pepper chromatin with less than two mitoses per HPFin NETG1. H&E, 40x view

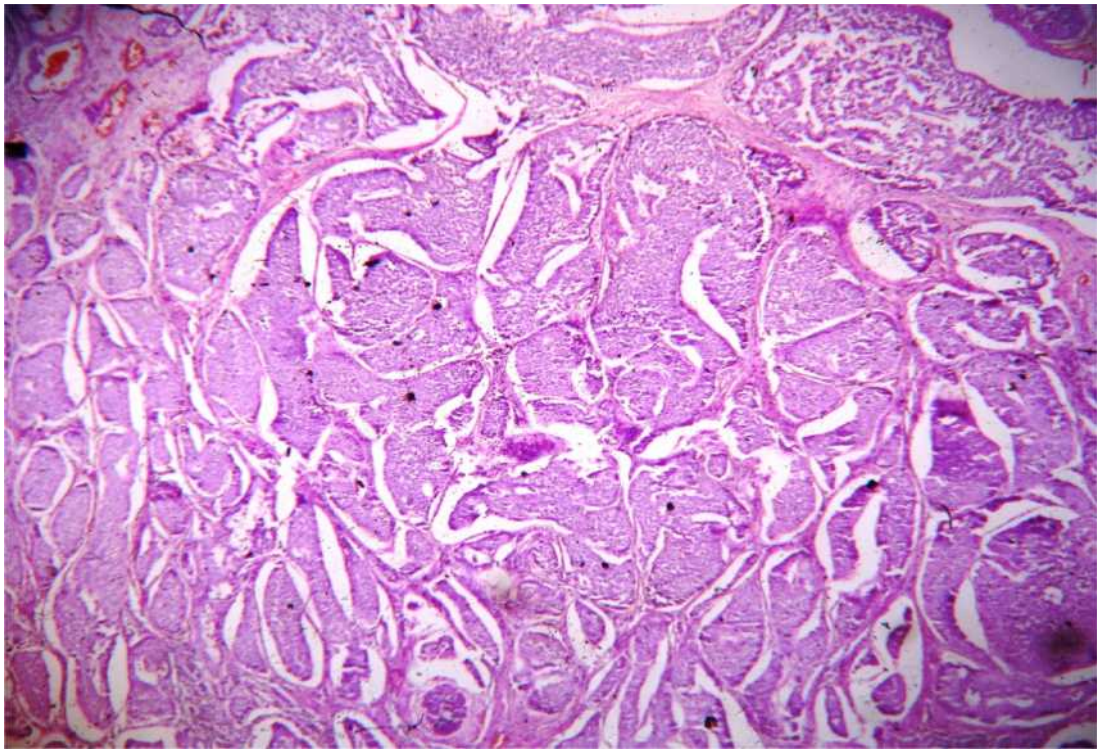


Fig 7: Neoplastic cells arranged in cords, nests, trabecular pattern in NET G2.10x view., H&E

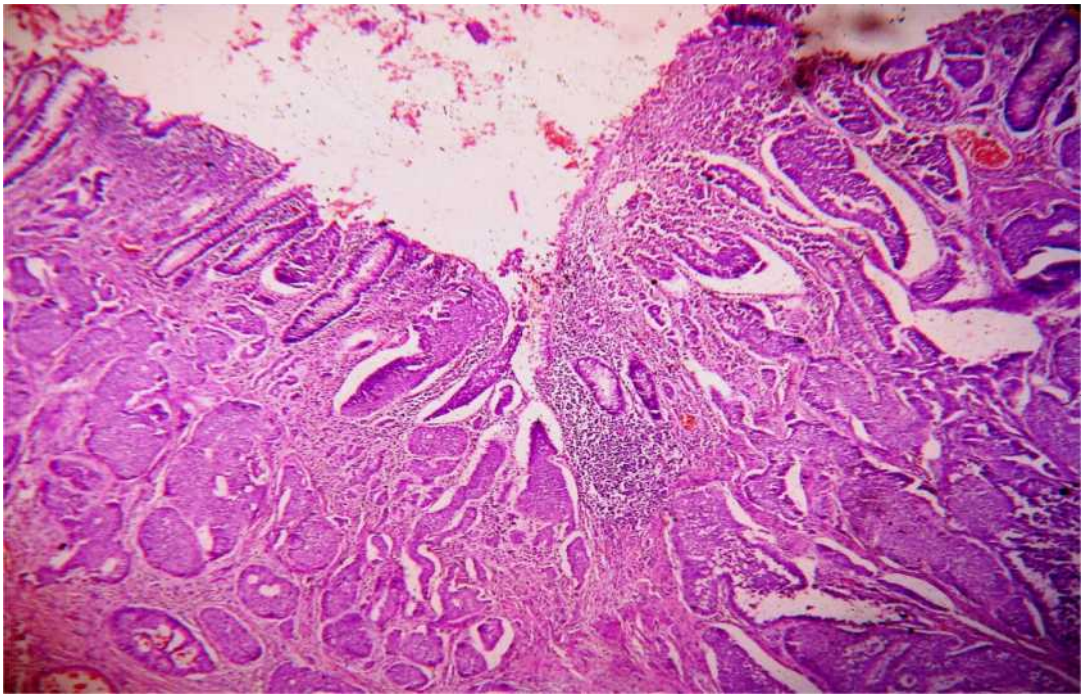


Fig 8: NET G2 showing ulcerated mucosa with neoplastic cells in sub mucosa 10x view, H&E

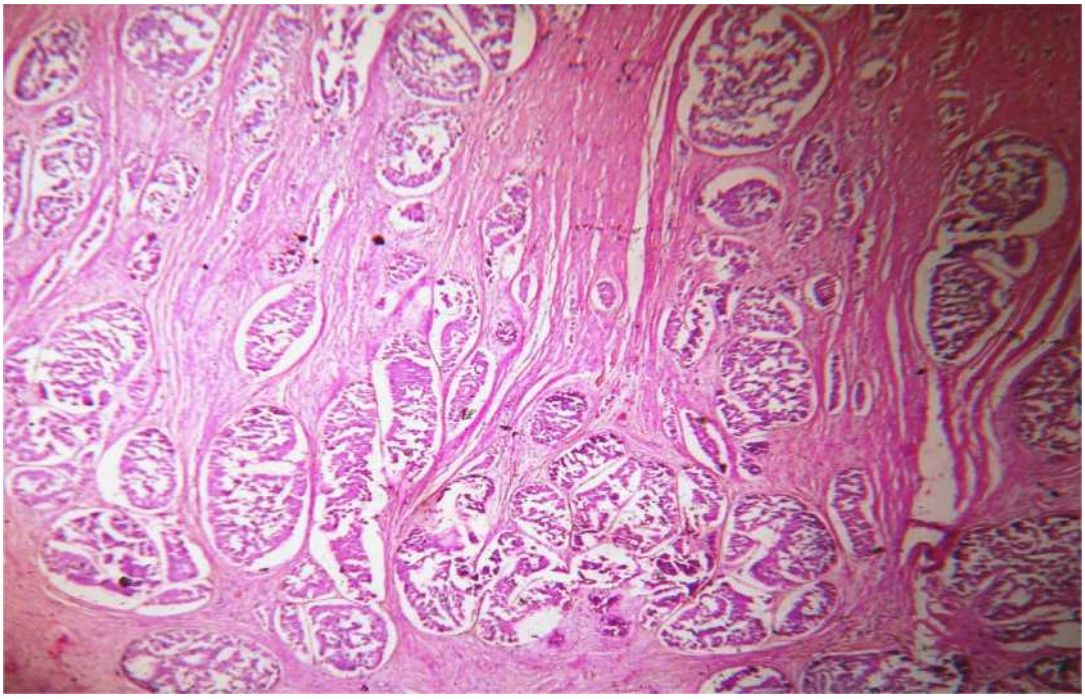


Fig 9: NET G2 invading the muscularis propria 10x view H&E

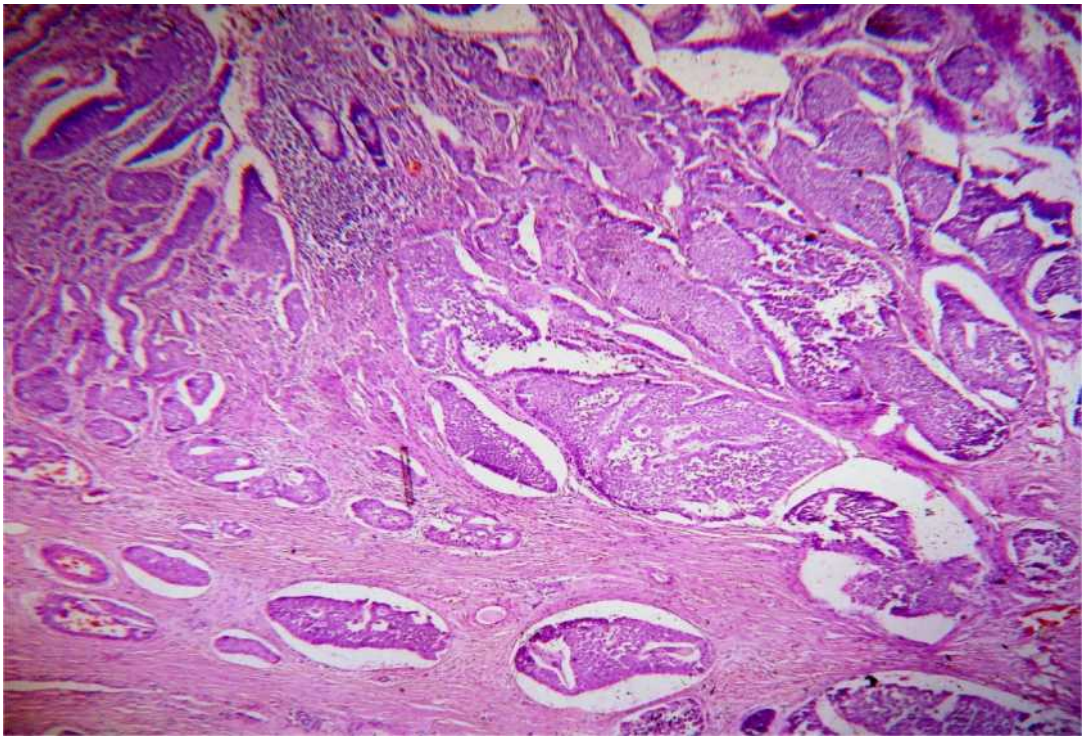


Fig 10 : NETG2 involving Muscularis propria. H&E, 10x view

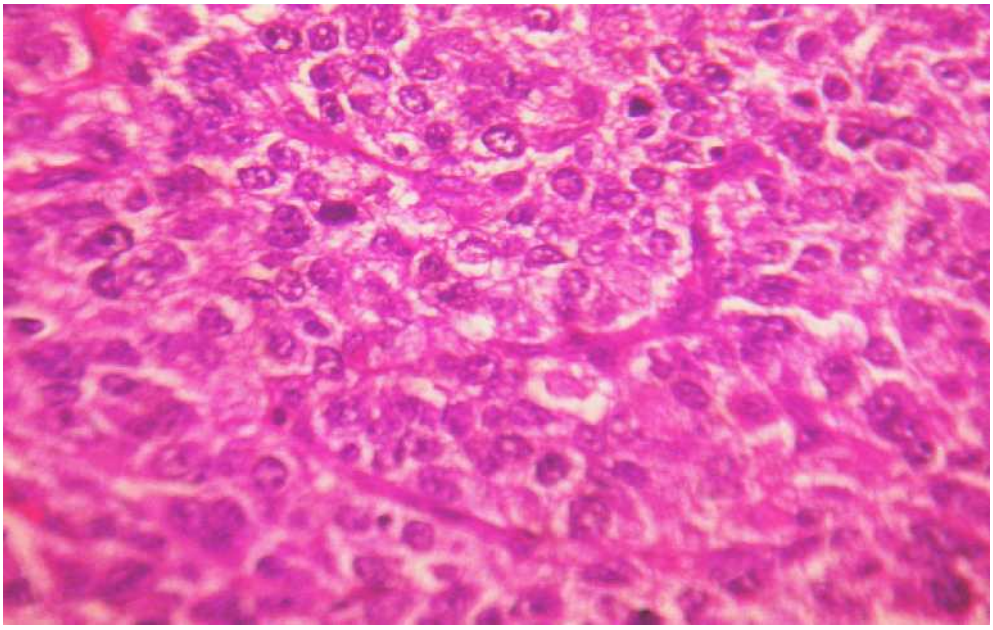


Fig 11: Cells with scant eosinophilic cytoplasm, uniform nucleus, finely stippled chromatin with mitoses 2-20 per HPF in NET G2. 45x view H&E

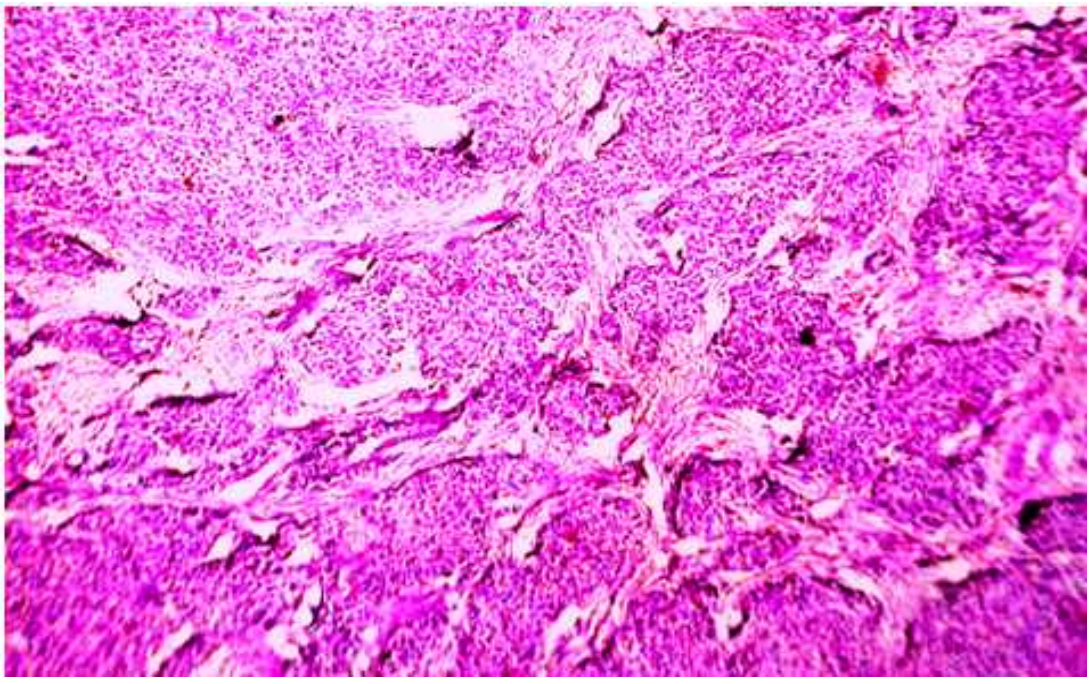


Fig 12: Cells in submucosa showing round cells with clear cytoplasm, round nuclei with clumped chromatin,>20 mitoses/10 HPF in NET G3.10x view. H&E

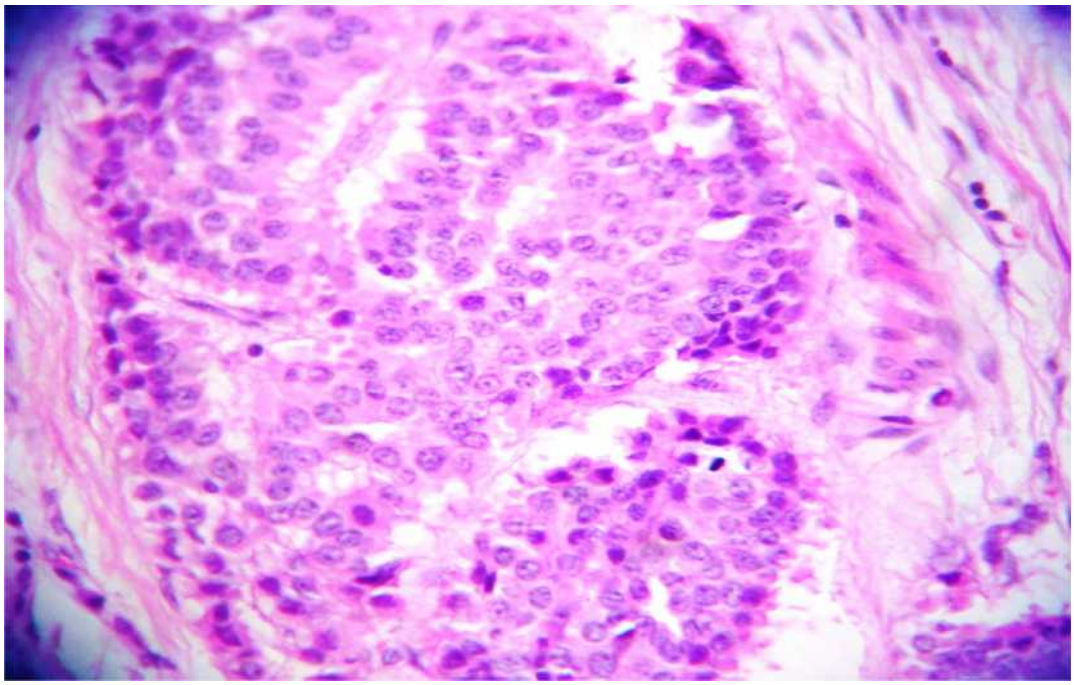


Fig 13: Round cells with clear cytoplasm, round nuclei with clumped chromatin,>20 mitoses/10HPF in NET G3.45x view H&E

Fig 14 : NSE positivity in NET G3.10x view.

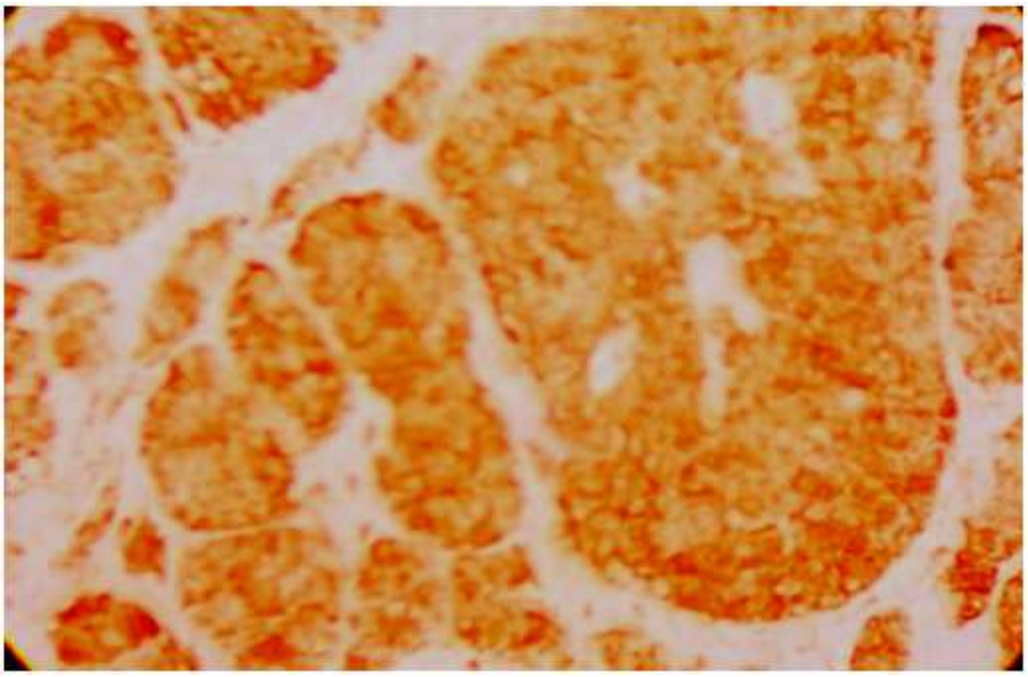


Fig 15 :40x view showing neuron specificc enolase positivity

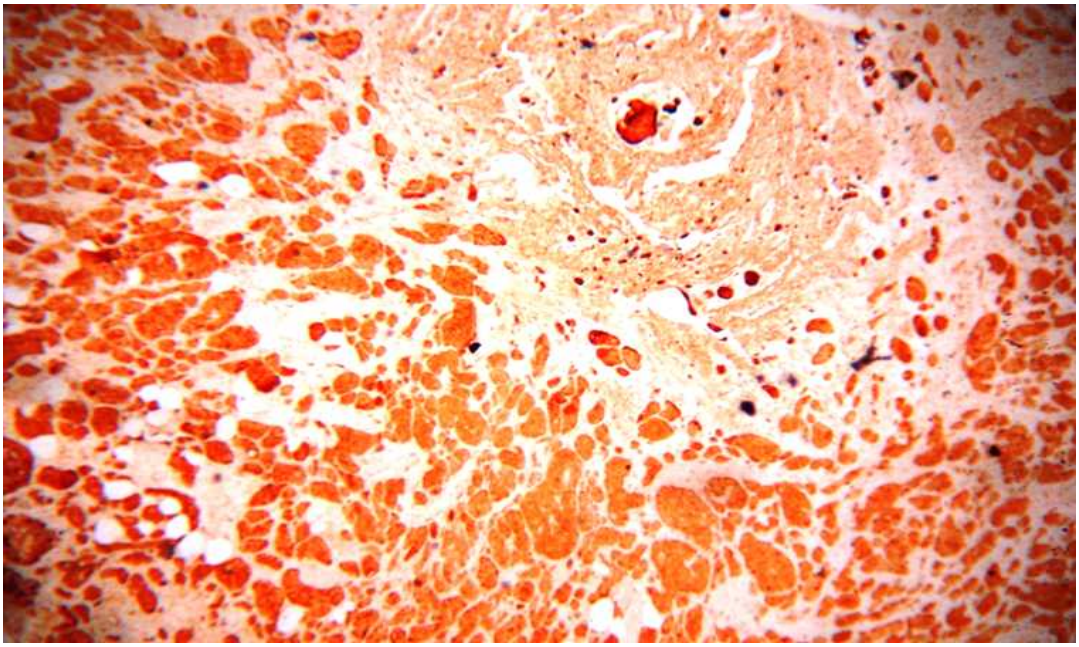


Fig 16: Scanner view showing synaptophysin positivity in well differentiated neuroendocrine tumor

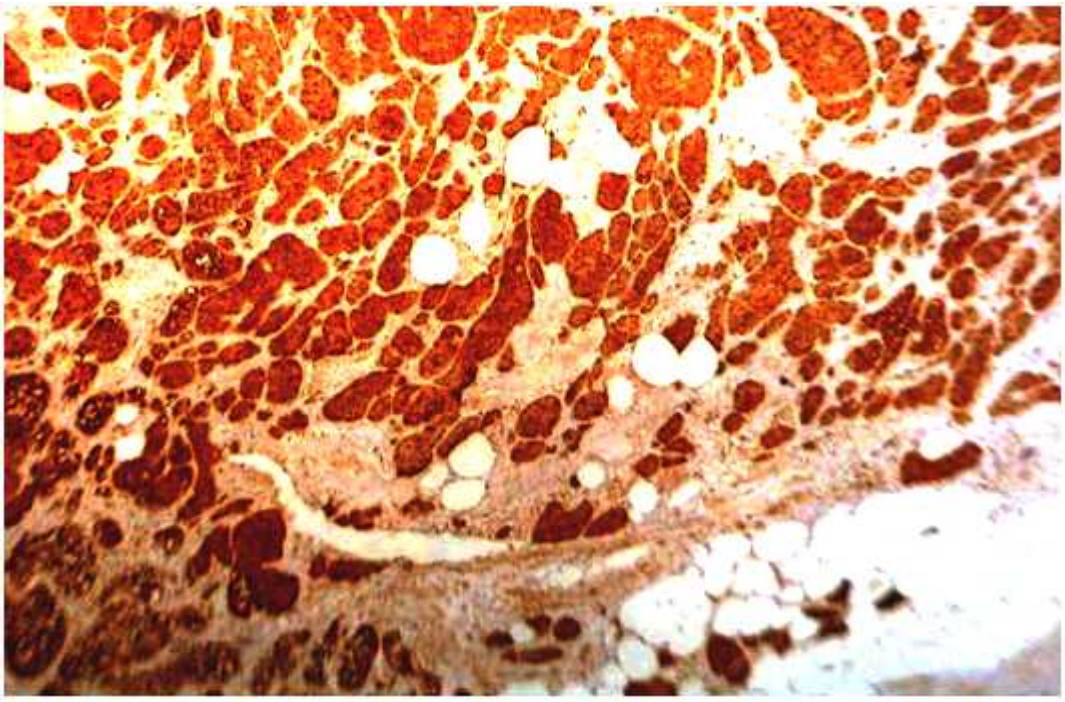


Fig 17:10x view showing synaptophysin positivity in a well differentiated neuroendocrine tumor

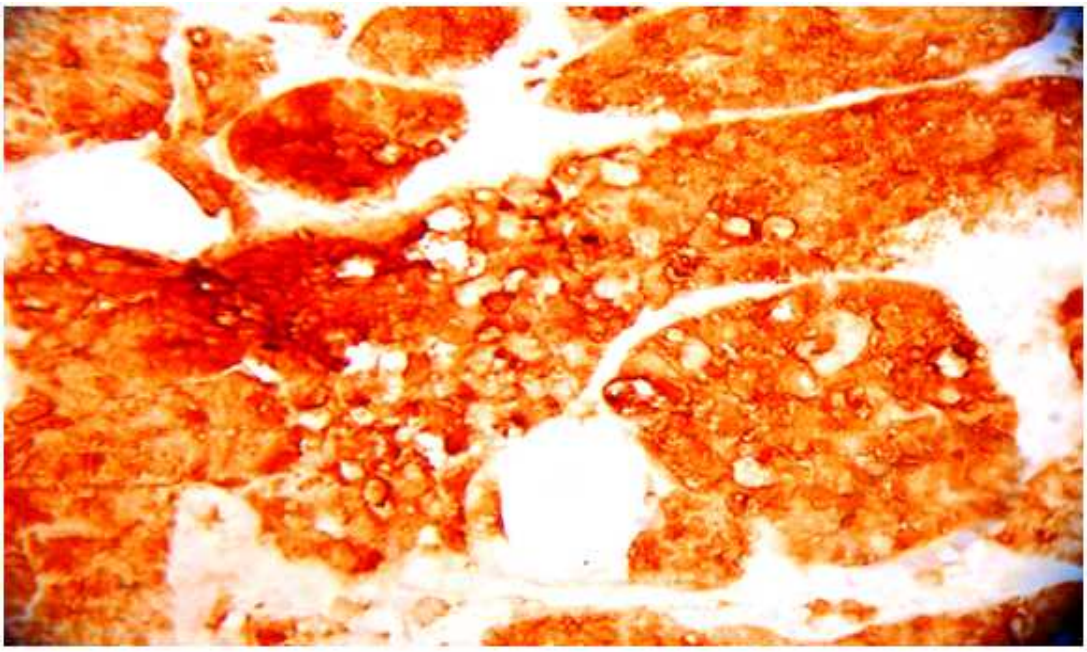


Fig 18:45x view showing synaptophysin positivity

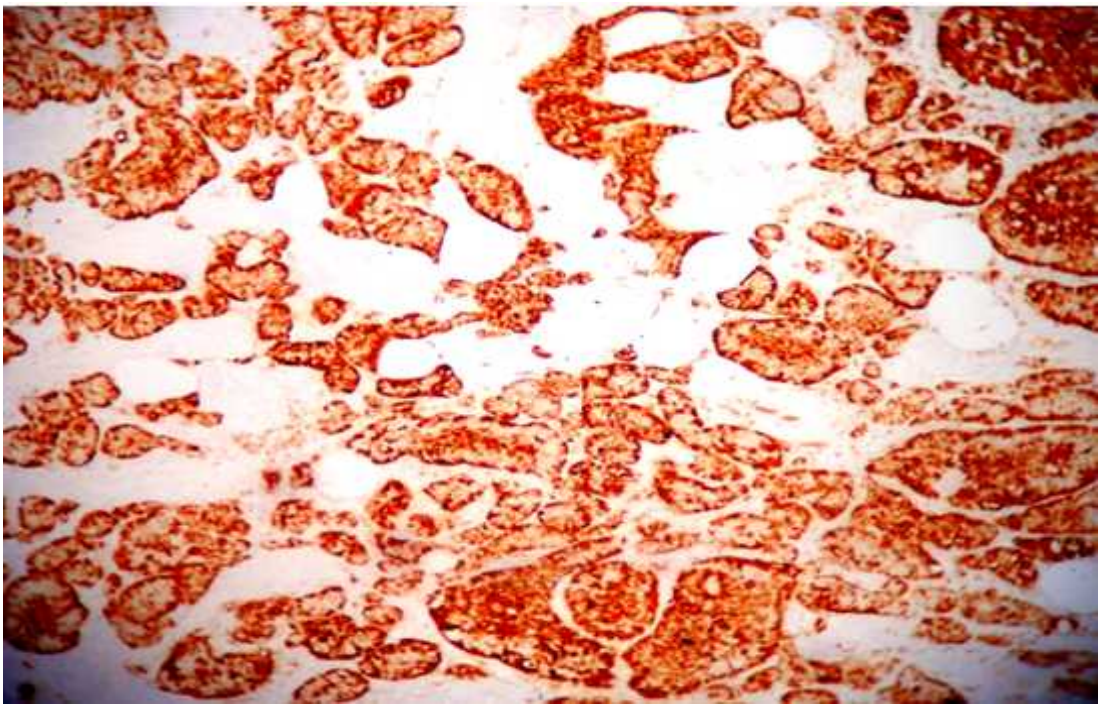


Fig 19:10x view showing chromogranin positivity

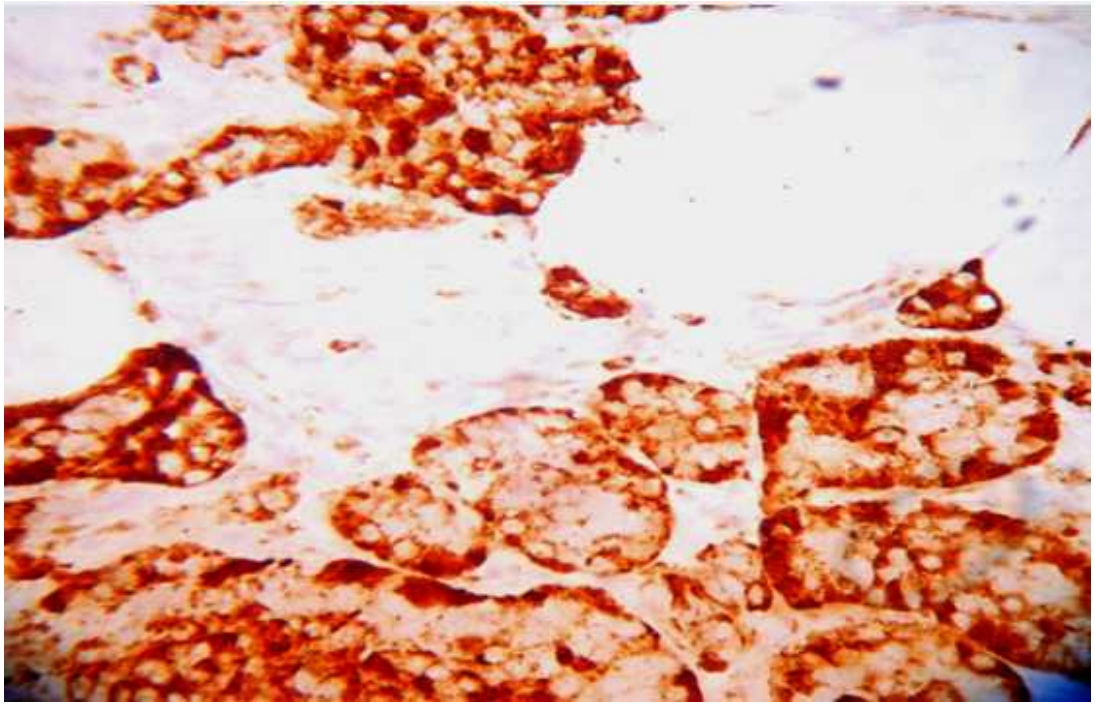


Fig 20:40x view showing chromogranin positivity

CHART 1

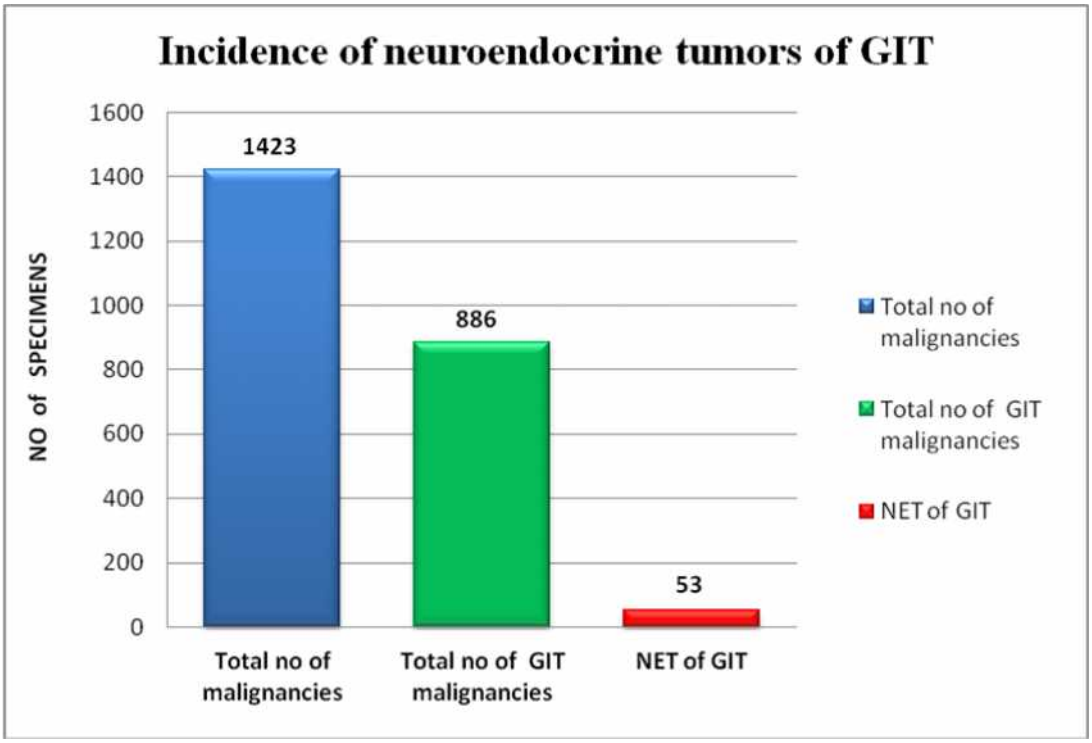

Incidence of neuroendocrine tumors of GIT
NO of SPECIMENS
1600
1400
1200
1000
800
600
400
200
0
1423
886
53
Total no of malignancies
Total no of GIT malignancies
NET of GIT
Total no of malignancies
Total no of GIT malignancies
NET of GIT

CHART 2

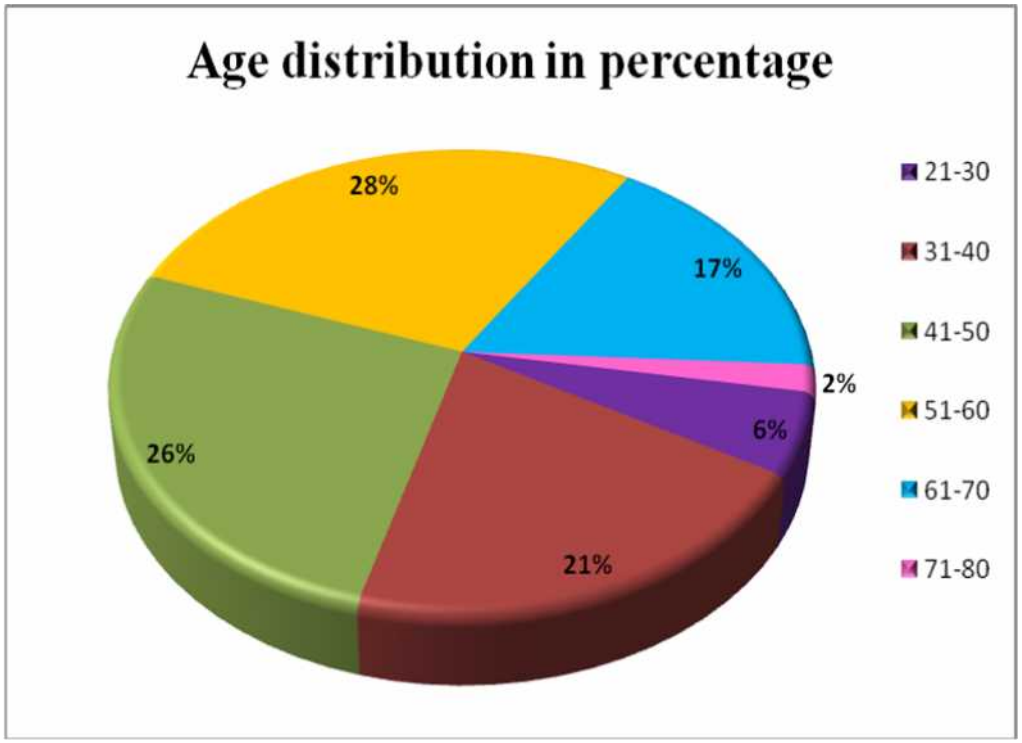

Age distribution in percentage
28%
17%
2%
6%
26%
21%
21-30
31-40
41-50
51-60
61-70
71-80

CHART 3

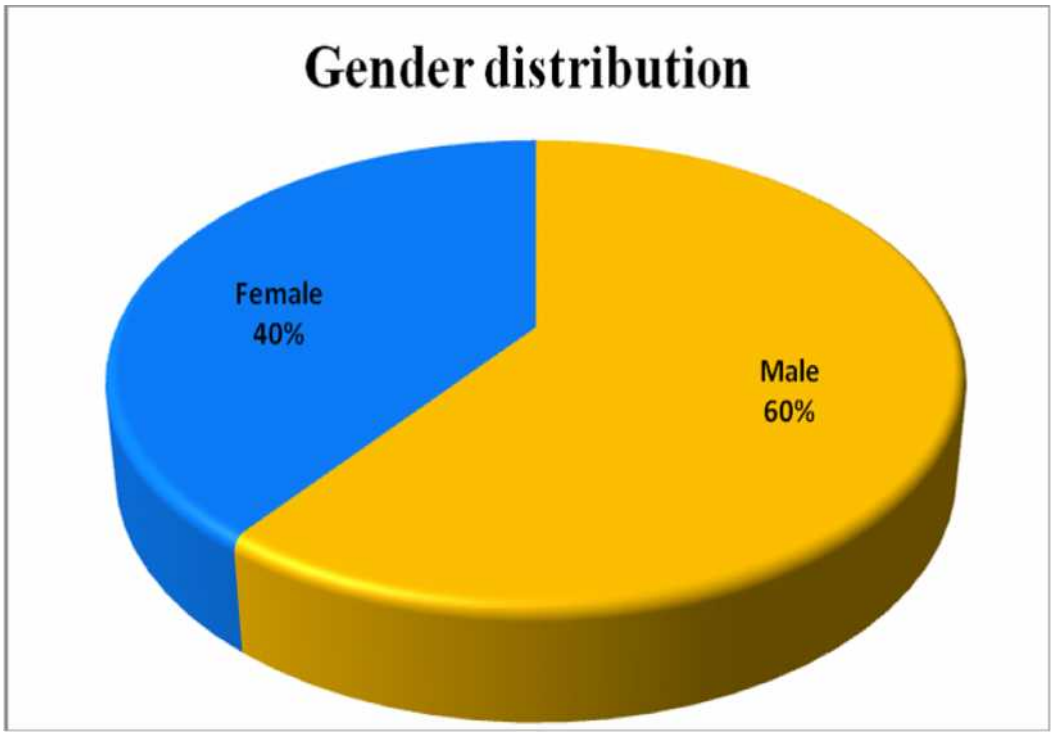


CHART 4

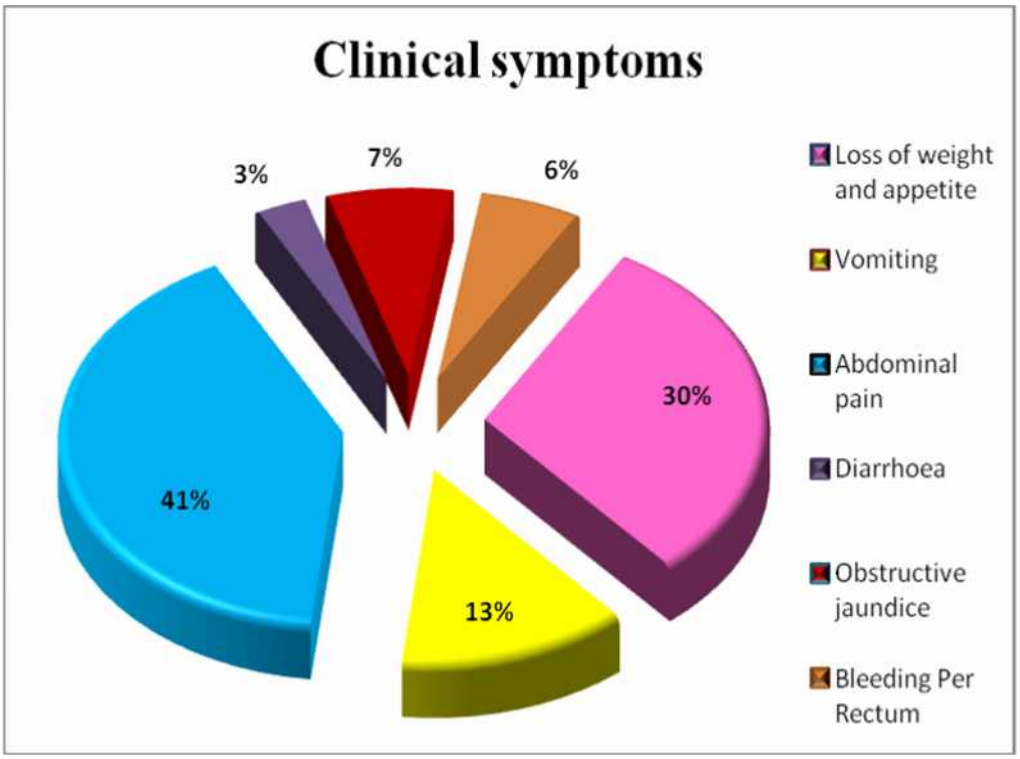

Clinical symptoms
3%
7%
6%
30%
41%
13%
Loss of weight and appetite
Vomiting
Abdominal pain
Diarrhoea
Obstructive jaundice
Bleeding Per Rectum

CHART 5

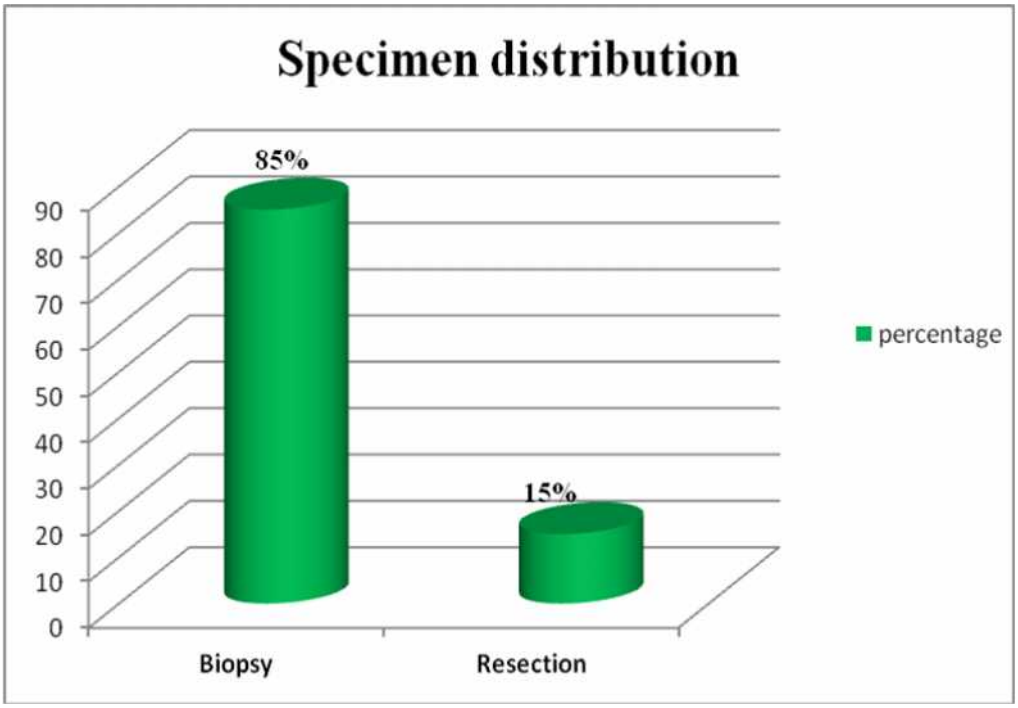

Specimen distribution
85%
15%
90
80
70
60
50
40
30
20
10
0
Biopsy
Resection
percentage

CHART 6

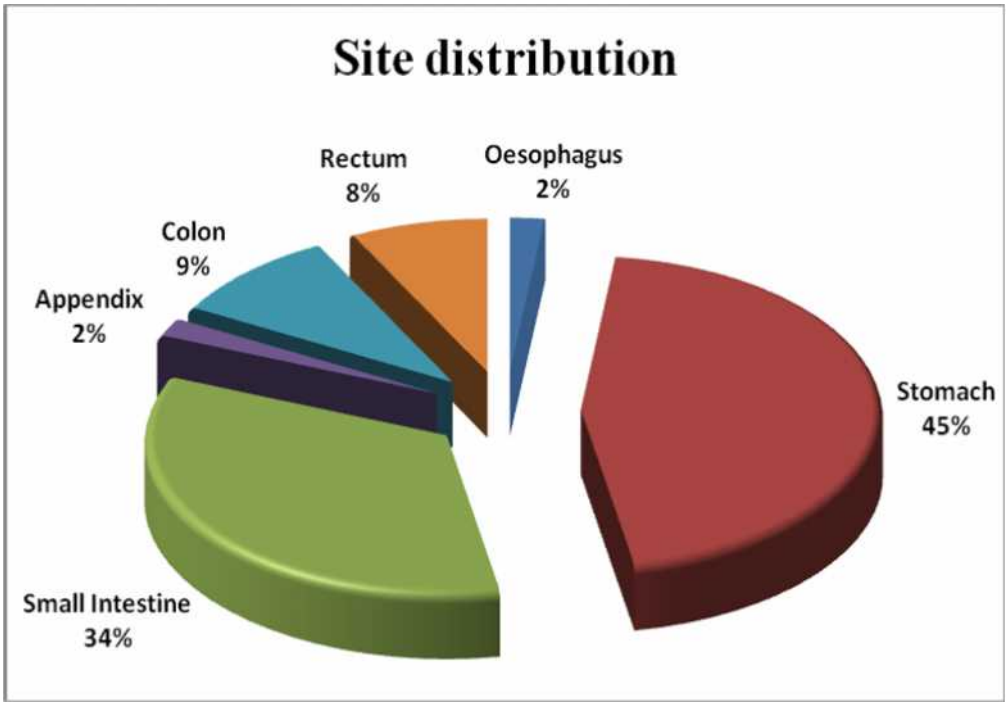

Site distribution
Rectum
8%
Oesophagus
2%
Colon
9%
Appendix
2%
Stomach
45%
Small Intestine
34%

CHART 7

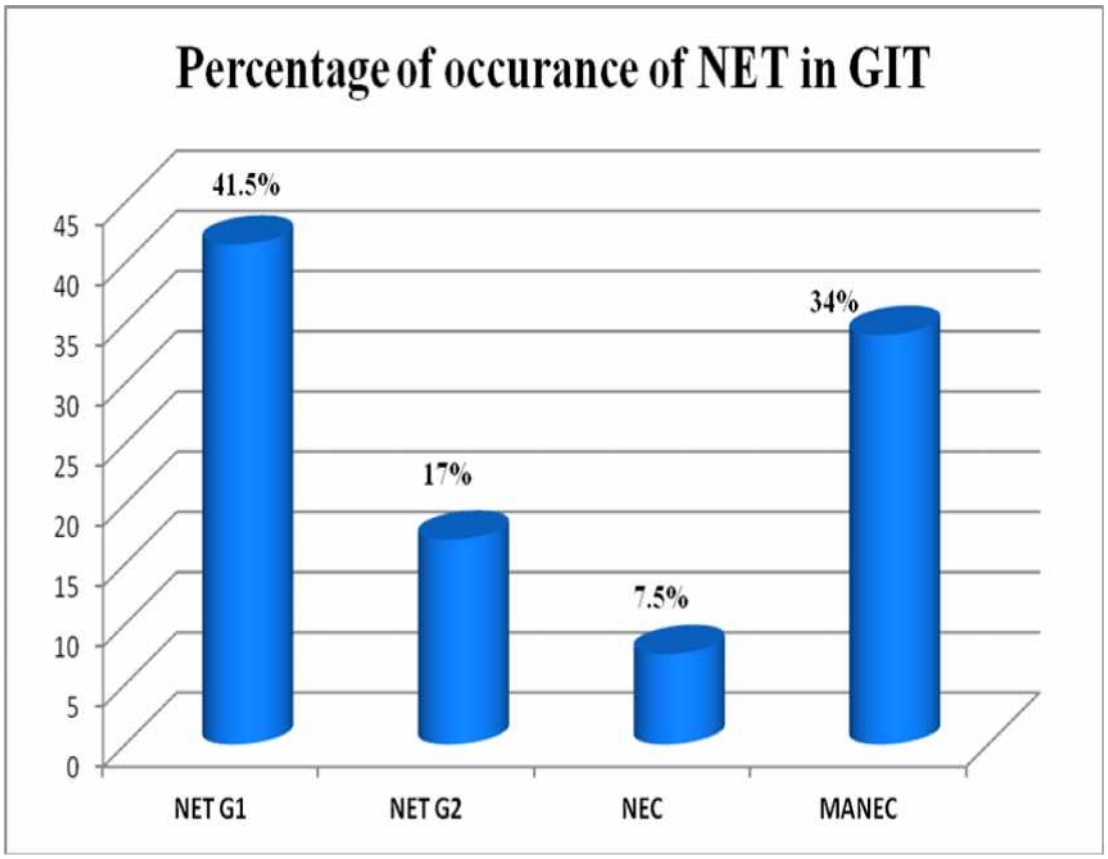
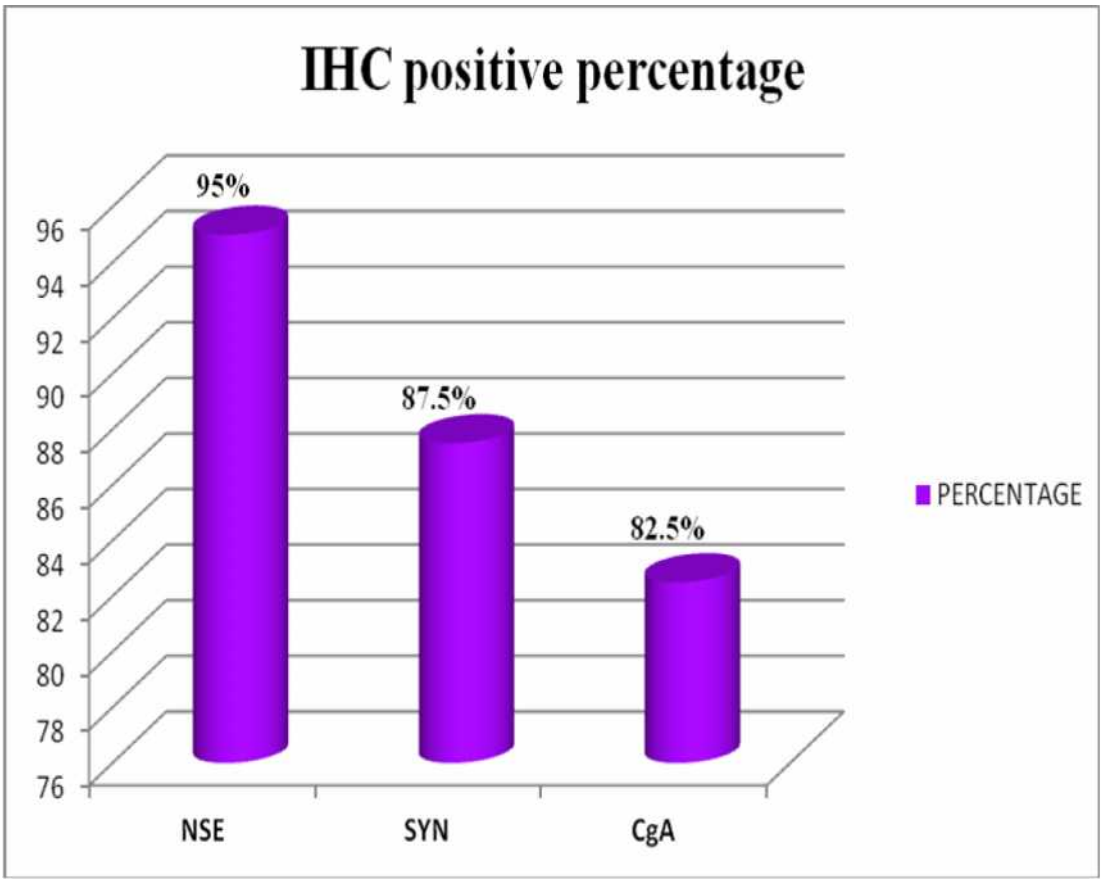

Percentage of occurance of NET in GIT
41.5%
17%
7.5%
34%
45
40
35
30
25
20
15
10
5
0
NET G1
NET G2
NEC
MANEC

CHART 8


IHC positive percentage
95%
87.5%
82.5%
96
94
92
90
88
86
84
82
80
78
76
NSE
SYN
CgA
PERCENTAGE

s.no	Biopsy no	Age	Sex	LOA, LOW	vomit	abd pain	diarrho	jaundice	Bleeding PR	secondaries	Carcinoid syn	Sample received	Site	Diag	NSE	SYN	CGA
1	52/08	71	M	1	0	1	0	0	0	0	0	MGWST	small intestine	NEC	1	0	0
2	169/08	61	M	0	1	1	0	0	0	0	0	MGWST	Stomach	NEC	1	1	1
3	752/08	50	F	1	0	1	0	0	0	0	1	MGWST	small intestine	NET G1	1	1	1
4	2641/08	40	F	0	0	1	1	0	0	0	0	MGWST	small intestine	NET G1	1	1	1
5	3123/08	61	M	0	0	1	0	0	0	0	0	Gastrectomy	Stomach	NET G2	1	0	1
6	3428/08	32	F	0	0	0	0	1	0	0	0	Whipple's proc	small intestine	MANEC	1	0	1
7	842/09	58	M	0	0	0	0	0	0	0	0	MGWST	small intestine	NET G1	1	1	1
8	R940/09	64	M	0	0	0	0	0	1	0	0	MGWST	Colon	MANEC	1	1	1
9	1164/09	60	F	1	0	1	0	0	0	0	0	Subtotal colectomy	Colon	NEC	1	1	0
10	1277/09	62	F	1	0	0	0	0	0	0	0	MGWST	Colon	NEC	0	0	1
11	2821/09	37	M	0	0	1	0	0	1	0	0	MGWST	Rectum	NET G1	1	1	0
12	2180/09	40	F	0	0	1	0	0	0	0	0	MGWST	Appendix	NET G1	1	1	1
13	R1169/09	50	F	0	0	0	0	0	0	0	0	MGWST	Stomach	NET G1	1	1	1
14	458/10	35	M	0	0	1	0	1	0	0	0	MGWST	small intestine	NET G1	1	1	1
15	R640/10	40	M	1	0	0	0	0	0	0	0	MGWST	small intestine	NET G1	1	1	1
16	685/10	25	M	1	0	1	1	0	0	1	0	MGWST	Rectum	MANEC	1	1	0
17	924/10	40	F	0	1	1	0	0	0	1	0	MGWST	Stomach	NET G2	1	1	1
18	1021/10	43	F	0	0	0	0	0	0	0	0	MGWST	Stomach	NET G2	1	1	0
19	R1045/10	70	M	1	0	1	0	0	0	0	0	MGWST	small intestine	NET G2	1	1	1
20	R1084/10	50	M	0	0	0	0	0	1	0	0	MGWST	Rectum	NET G2	1	1	1
21	1310/10	60	M	0	1	1	0	0	0	0	0	MGWST	Stomach	MANEC	0	1	0
22	1541/10	30	M	0	0	1	0	0	0	0	0	MGWST	Stomach	NET G1	1	1	1
23	1430/10	45	M	1	0	0	0	0	0	0	0	MGWST	Stomach	NET G2	1	1	1
24	1634/10	45	M	1	0	1	0	0	0	0	0	MGWST	Stomach	NET G2	1	1	1
25	1890/10	51	F	0	0	0	0	0	0	0	0	MGWST	oesopagus	NET G1	1	1	1
26	2035/10	60	F	1	0	0	0	0	0	0	0	MGWST	Stomach	MANEC	1	0	0
27	107/11	60	M	0	0	1	0	0	0	0	1	MGWST	Stomach	NET G1	1	1	1
28	404/11	62	F	0	0	1	0	1	0	0	0	MGWST	small intestine	NET G1	1	1	1
29	446/11	70	M	1	0	1	0	1	0	0	0	MGWST	Stomach	NET G1	1	1	1
30	R798/11	55	M	0	0	0	0	0	0	0	0	MGWST	small intestine	NET G1	1	1	1
						0= absent		1=present				0=neg	1=pos				

s.no	Biopsy no	Age	Sex	LOA, LOW	vomit	abd pain	diarrho	jaundice	Bleeding PR	secondaries	Carcinoid syn	Sample received	Site	Diag	NSE	SYN	CGA
31	R1076/11	50	F	0	0	1	0	1	0	0	0	MGWST	small intestine	NET G1	1	1	1
32	R1202/11	54	F	1	0	0	0	0	0	0	0	total gastrectomy	Stomach	NET G1	1	1	1
33	1448/11	55	F	0	0	0	0	0	0	0	0	MGWST	small intestine	MANEC	1	1	1
34	1733/11	59	M	0	1	1	0	0	0	0	0	MGWST	Stomach	MANEC	1	1	1
35	1775/11	49	F	0	1	1	0	0	0	0	0	MGWST	Stomach	MANEC	1	1	1
36	2410/11	57	M	1	0	0	0	0	0	0	0	MGWST	Stomach	MANEC	1	1	1
37	2441/11	55	F	0	0	0	0	0	0	0	0	MGWST	small intestine	NET G1	1	1	1
38	563/12	47	M	1	0	0	0	0	0	0	0	sub gastrectomy	Stomach	MANEC	1	1	1
39	1339/12	45	M	0	1	1	0	0	0	0	0	MGWST	Stomach	NET G1	1	1	1
40	1359/12	54	M	1	0	0	0	0	0	0	0	MGWST	Stomach	NET G1	1	1	1
41	650/08	48	M	0	0	0	0	0	0	1	0	Hemicolectomy	Colon	MANEC	ND	ND	ND
42	R1104/08	70	F	1	0	0	0	0	0	0	0	MGWST	small intestine	MANEC	ND	ND	ND
43	2437/08	27	F	0	0	0	0	0	0	0	0	Ileal resection	small intestine	NET G2	ND	ND	ND
44	2842/08	40	M	1	0	0	0	0	0	0	0	MGWST	small intestine	NET G1	ND	ND	ND
45	3092/08	50	M	0	0	0	0	0	1	0	0	APR	Rectum	NET G1	ND	ND	ND
46	460/09	43	M	1	0	1	0	0	0	0	0	MGWST	Stomach	NET G1	ND	ND	ND
47	R262/09	40	M	1	0	1	0	0	0	0	0	MGWST	small intestine	MANEC	ND	ND	ND
48	1007/09	37	M	0	1	1	0	0	0	0	0	MGWST	Stomach	NET G2	ND	ND	ND
49	2139/09	65	M	0	1	1	0	0	0	0	0	MGWST	Stomach	MANEC	ND	ND	ND
50	1690/10	52	M	0	0	1	0	0	0	0	0	MGWST	Colon	MANEC	ND	ND	ND
51	146/12	38	F	1	0	0	0	0	0	0	0	MGWST	small intestine	MANEC	ND	ND	ND
52	402/12	49	M	0	1	1	0	0	0	0	0	MGWST	Stomach	MANEC	ND	ND	ND
53	689/11	56	F	1	0	0	0	0	0	0	0	MGWST	Stomach	MANEC	ND	ND	ND
						0= absent		1=present				0=neg	1=pos		ND=not done		

ANNEXURE

Tumour, Node, Metastasis (TNM) staging system for Gastric Neuroendocrine Tumors:

(T)	Primary tumor
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ/dysplasia (tumor size < 0.5 mm), confined to mucosa
T1	Tumor invades lamina propria, or submucosa and ≤ 1cm insize
T2	Tumor invades muscularis propria or > 1 cm in size
T3	Tumor penetrates subserosa
T4	Tumor invades visceral peritoneum (serosal) or other organs or adjacent structures

For any T, (m) should be added for multiple tumors

Regional lymphnodes (N)

Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis

Distant metastasis (M)

M0	No distant metastasis
M1	Distant metastasis

STAGE GROUPING

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	Any T	N1	M0
Stage IV	Any T	Any N	M1

Tumour, Node, Metastasis (TNM) staging system for Neuroendocrine Tumors duodenum/ampulla/jejunum/ileum:

(T)	Primary tumor
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor invades lamina propria,or submucosa and ≤ 1cm in size(Small intestinal tumors);tumor ≤ 1 cm (Ampullary tumors)
T2	Tumor invades muscularis propria or > 1 cm in size(Small intestinal tumors));tumor > 1 cm (Ampullary tumors)
T3	Tumor invades through the muscularis propria into the subserosal tissue without penetration of the overlying serosa(Jejunal or Ileal tumors) or invades Pancreas or Retroperitoneum(Ampullary or Duodenal tumors) or into non retroperitonealized tissues
T4	Tumor invades visceral peritoneum (serosa) or invades other organs

For any T, (m) should be added for multiple tumors

Regional lymph nodes (N)

Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis

Distant metastasis (M)

M0	No distant metastasis
M1	Distant metastasis

STAGE GROUPING

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
Stage IIB	T3	N0	M0
Stage IIIA	T4	N0	M0
Stage IIIB	Any T	N1	M0
Stage IV	Any T	Any N	M1

[TNM classification of malignant tumours (7th edition). International Union Against Cancers http://www.uiccorg/tnm 2010]

PROFOMA

SERIAL NO : **BIOPSY NO:** **DATE:**

NAME :

AGE:

SEX:

CLINICAL PRESENTATION:

INVESTIGATIONS:

CLINICAL DIAGNOSIS:

GROSS FINDINGS:

HISTOPATHOLOGICAL EXAMINATION:

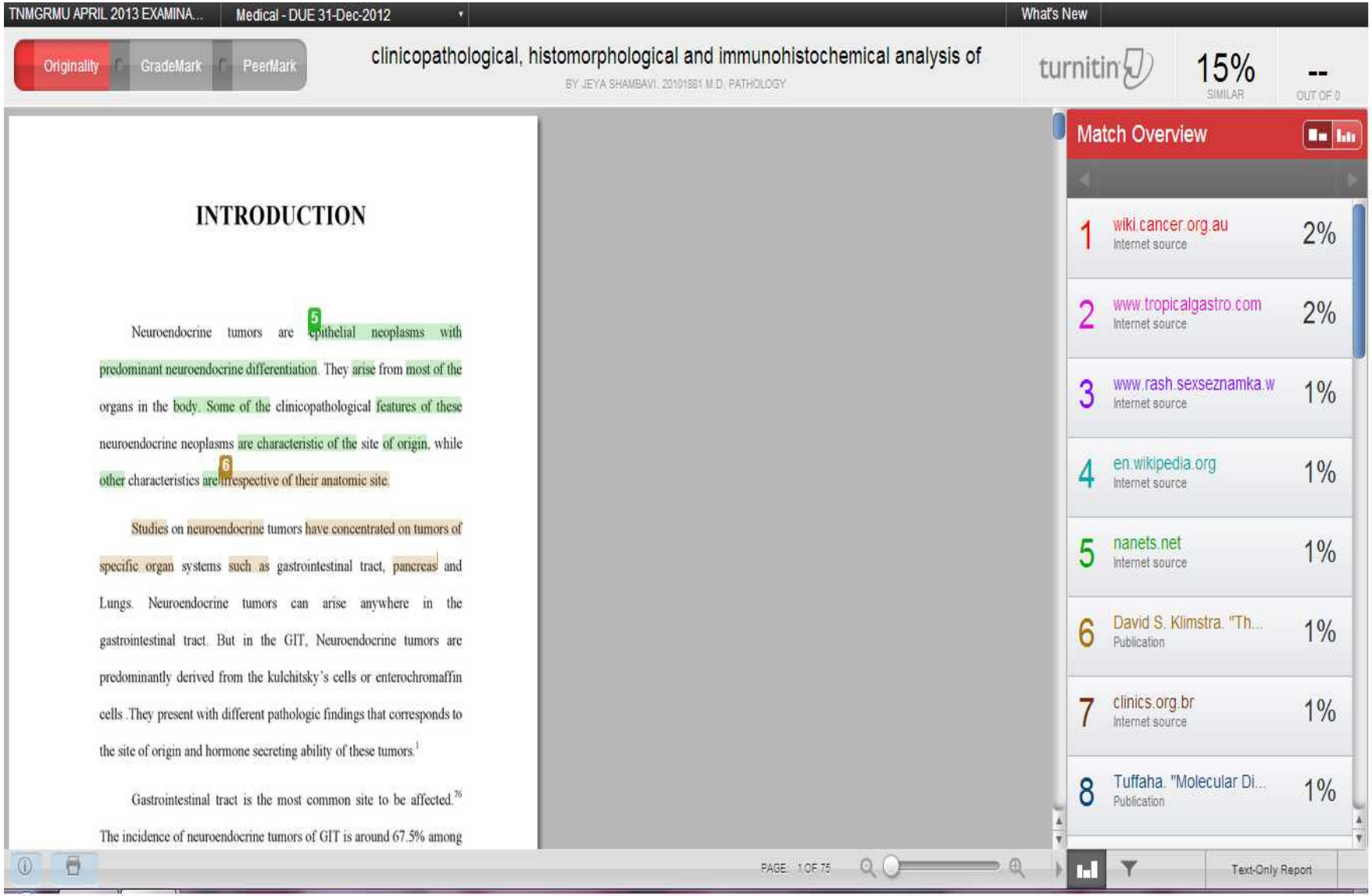
IMMUNOHISTOCHEMISTRY:

IMPRESSION:

ABSTRACT

Neuroendocrine tumors are relatively rare neoplasms that arise in various organs and share many common pathological features. In the year 2010 WHO graded Neuroendocrine tumors based on mitotic rate and Ki-67 index into NET G1, NET G2, NET G3 and Mixed adenoneuroendocrine carcinomas. The objectives of this study were to evaluate clinicopathological, histomorphological and immunohistochemical features of neuroendocrine tumors of the gastrointestinal tract. In this study, a total of 53 specimens received between 2008 and 2012 diagnosed as having neuroendocrine tumors of GIT were analysed for histopathological and immunohistochemical expression. **Results**: In our study neuroendocrine tumors of git were more common in males. The most common anatomical location was stomach. The median age at diagnosis was fifty. Most of the tumors were NET G1.We found MANEC to have a higher incidence. The positivity rates for NSE, Synaptophysin and chromogranin A was 97.5%, 87.5% and 82.5% respectively. **Conclusion**: The incidence of neuroendocrine tumors is on the rise. Immunohistochemical confirmation of histopathological diagnosis is important to distinguish NETs from other neoplasms and to give a definitive diagnosis.

Keywords:Neuroendocrine tumors,Carcinoids,immunohistochemistry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INTRODUCTION
Neuroendocrine tumors are epithelial neoplasms with predominant neuroendocrine differentiation. They arise from most of the organs in the body. Some of the clinicopathological features of these neuroendocrine neoplasms are characteristic of the site of origin, while other characteristics are irrespective of their anatomic site.
Studies on neuroendocrine tumors have concentrated on tumors of specific organ systems such as gastrointestinal tract, pancreas and Lungs. Neuroendocrine tumors can arise anywhere in the gastrointestinal tract. But in the GIT, Neuroendocrine tumors are predominantly derived from the kulchitsky's cells or enterochromaffin cells .They present with different pathologic findings that corresponds to the site of origin and hormone secreting ability of these tumors.[1]
Gastrointestinal tract is the most common site to be affected.[76] The incidence of neuroendocrine tumors of GIT is around 67.5% among
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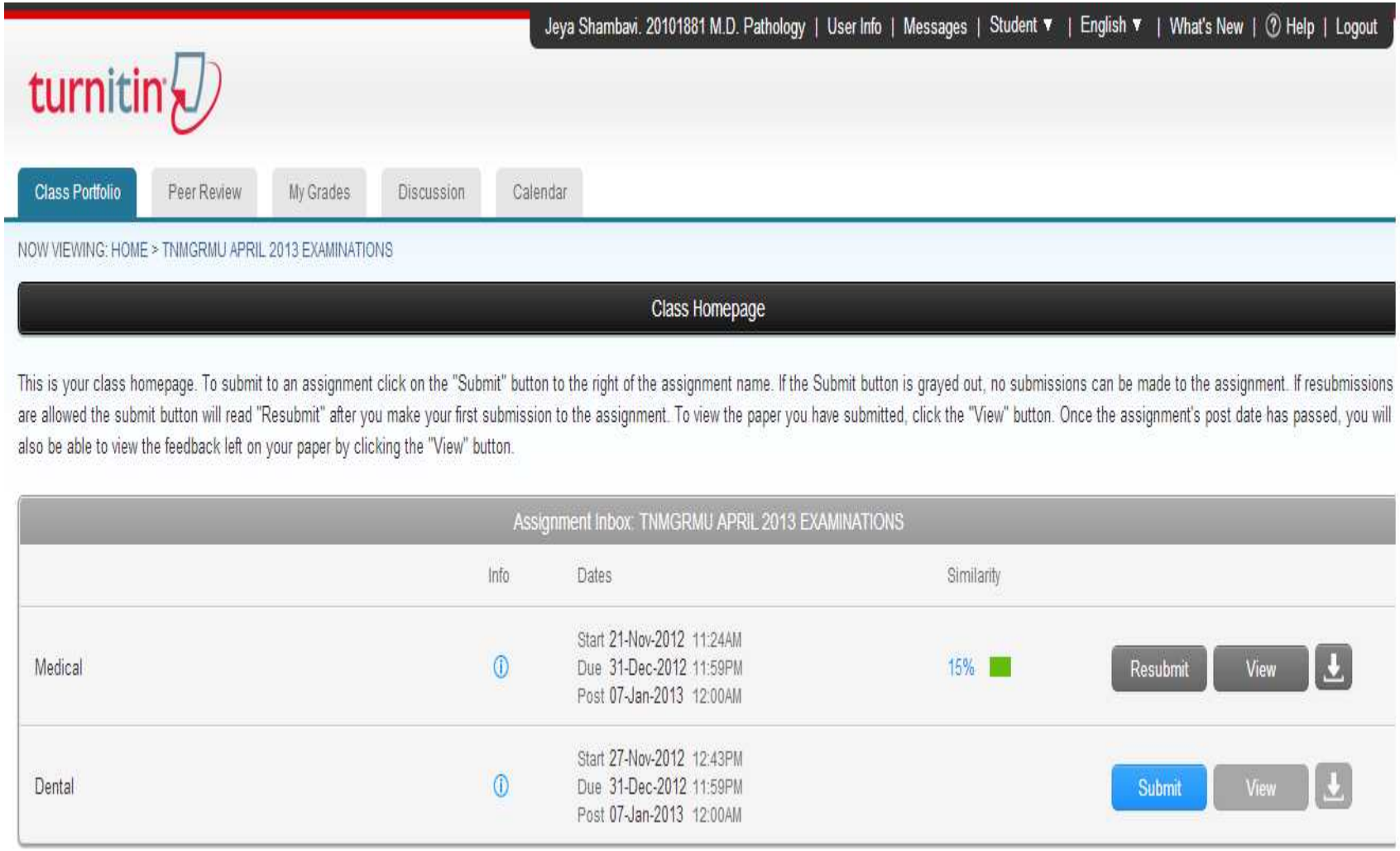
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