CD117, DOG1 AND KI67 EXPRESSION IN GASTROINTESTINAL STROMAL TUMOURS

Lata Manchala[1], Devender Reddy Kotta[2], Shrinivas Bheemrao Somalwar[3], Ezhil Arasi Nagamuthu[4]

[1]Postgraduate Student, Department of Pathology, Osmania Medical College, Hyderabad.
[2]Associate Professor, Department of Pathology, Osmania Medical College, Hyderabad.
[3]Assistant Professor, Department of Pathology, Osmania Medical College, Hyderabad.
[4]Professor and HOD, Department of Pathology, Osmania Medical College, Hyderabad.

ABSTRACT

BACKGROUND

GIST is a subset of mesenchymal tumours arising from interstitial cells of Cajal. GIST has become very important since the availability of specific pathogenesis targeted treatment with the KIT/PDGFRA tyrosine kinase inhibitor, imatinib mesylate.

MATERIALS AND METHODS

It is a prospective and retrospective study. The study was conducted at the Upgraded Department of Pathology, Osmania General Hospital, Hyderabad, from 2013-2015 on 40 tumour samples. Immunohistochemistry using CD117, DOG1 and Ki67 was done on all cases along with routine histopathological assessment on H and E stained slides.

RESULTS

The immunopositivity of various markers is observed in different grades depending upon the tumour grade and locations.

CONCLUSION

CD117, DOG1 and Ki67 are important key diagnostic and prognostic biomarkers in the assessment of GIST across all the mesenchymal tumours of GIT.

KEYWORDS

GIST, DOG1, c-Kit, PDGFRA.

HOW TO CITE THIS ARTICLE: Manchala L, Kotta DR, Somalwar SB, et al. CD117, DOG1 and Ki67 expression in gastrointestinal stromal tumours. J. Evid. Based Med. Healthc. 2017; 4(75), 4417-4422. DOI: 10.18410/jebmh/2017/880

BACKGROUND

Gastrointestinal Stromal Tumours (GISTs) are a subset of mesenchymal tumours arising from interstitial cells of Cajal present in muscularis propria layer of GI tract. The demonstration of mutations in the c-Kit gene and PDGFRA in GISTs has become very important since the availability of specific pathogenesis targeted treatment with the KIT/PDGFRA tyrosine kinase inhibitor imatinib mesylate.[1]

Gastrointestinal stromal tumours have a wide clinicopathologic spectrum. Most patients are adults with a median age of 50-60 years. Microscopic features are site dependent, but most GISTs are spindle cell tumours and a minority has epithelioid, mixed and rarely pleomorphic histology.

In the present study, clinicopathological spectrum of GIST along with immunohistochemical expression of c-Kit, DOG1 and Ki67 in these tumours is studied to differentiate them from other spindle cell tumours of gastrointestinal tract, which mimics GIST morphologically. Specific diagnosis of GIST enables delivery of potentially lifesaving treatment to the right patient with targeted therapy by tyrosine kinase inhibitors such as imatinib and sunitinib.

DOG1 is found to be more sensitive and specific than c-Kit in the present study as demonstrated by earlier studies by Espinosa et al[2] because DOG1 expression was not related to the mutational status.

Ki67 index is an independent prognostic factor Ki67 is associated nuclear proliferation antigen.[3] High-risk patients with Ki67 index >8% showed a poorer prognosis even with imatinib adjuvant therapy.

Even when both c-Kit and DOG1 expression is negative (approximately 2%) it is legitimate to make the diagnosis of GIST on morphological grounds,[4] but in order to support the patient with targeted therapy, mutational analysis should be carried out at higher centres. As mutational analysis is costly and needs specialised equipment, it is a major limitation to the present study.

Aim and Objective

To study the expression of CD117, DOG1 and Ki67 in gastrointestinal stromal tumours and to evaluate its utility as a prognostic marker for gastrointestinal stromal tumours.

MATERIALS AND METHODS

It is a prospective and retrospective study. The study was conducted at the Upgrade Department of Pathology, Osmania General Hospital, Hyderabad, from 2013-2015.

Financial or Other, Competing Interest: None.
Submission 14-08-2017, Peer Review 21-08-2017,
Acceptance 29-08-2017, Published 15-09-2017.
Corresponding Author:
Dr. Devender Reddy Kotta,
Associate Professor, Department of Pathology,
Osmania Medical College, Hyderabad.
E-mail: kndnreddy@gmail.com
DOI: 10.18410/jebmh/2017/880

Available clinical data including patient's age, sex, clinical presentation, physical examination, investigations (haematological, radiological, endoscopic) surgical findings were reviewed and analysed.

Inclusion Criteria

1. All the surgically-resected specimens with the tumour site in or adjacent to the gastrointestinal tract, mesentery, omentum or retroperitoneum.
2. Spindled and/or epithelioid morphologic patterns compatible with GIST.

Resected specimens were fixed in an adequate volume of 10% neutral buffered formalin for 24-48 hours, then the specimens were examined and the site of the tumours was recorded. Tumour size and maximum diameter was measured and the distance to the closest surgical and circumferential resected margins was recorded. Cut section of the tumour with areas of necrosis, haemorrhage or myxoid change were noted. The following tissue blocks were then taken.

- Margin- Surgical and circumferential resection margin.
- Tumour- Based on tumour size and heterogeneity, sufficient blocks of the tumour are taken to ensure that all macroscopically different areas were sampled (e.g. areas of haemorrhage or myxoid change). One block per cm of the tumour diameter is recommended. A block containing tumour and adjacent mucosa/muscularis propria is taken for the purpose of an internal control for immunohistochemistry.
- One block of normal background mucosa.
- Lymph nodes.

Macroscopic Assessment

- Specimen type (partial or complete resected specimen).
- Site and size of tumour.
- Resection margins - Distance of tumour to nearest surgical margins.

Microscopic Assessment

After the gross examination, the representative tissues were subject to histopathologic processing and paraffin embedding. 4-6 µm thick sections were cut were stained routine with H and E.

The following parameters were noted on microscopic examination.

Tumour Type

GISTs maybe of spindle cell type, epithelioid type or mixed type.

Mitotic Count

Mitotic count should be expressed as the number of mitoses per 50 high-power fields maybe equivalent to 5 mm^2. The count is done from the areas with the highest mitotic activity. Atypical mitotic figures are uncommon in GISTs.

Nuclear atypia, necrosis, ulceration of mucosa and extent of invasion was also noted.

Immunohistochemistry

The most representative blocks of all cases were selected and studied for the expression of DOG1, CD117, Ki67 index along with SMA, vimentin and pan CK for the differential diagnosis in required cases. Appropriate positive and negative controls were used for each antibody. The CD117 staining reaction was interpreted as diffuse cytoplasmic, membranous and perinuclear or dot-like positivity as in Figure 5 (a-d). DOG1 staining showed strong diffuse cytoplasmic positivity Figure 6. Ki67 showed nuclear positivity as shown in Figure 7 (a, b).

Antibody	Clone	Dilution	Source
CD117 (KIT)	Rabbit polyclonal	1:600	DAKO
DOG1	DOG1. 1 mouse monoclonal	Pre-diluted	Pathnsitu
Ki67	MIB-1	Pre-diluted	DAKO

Table 1. Primary Antibodies Used in the Study and Sources

Ki67 immunostaining was graded as follows-
Each slide was scanned at low power and area with the highest density of labeled nuclei was selected for counting at a high magnification (40x). An average of 1000 cells was counted. Based on cut-off value of LI >10% tumours were divided into 2 groups; low grade (≤10%) and high grade (>10%) Ki67 expression (Figure 7(a, b)).

OBSERVATION AND RESULTS

The present study comprises of 40 cases and the following observations were made.

Age Distribution

The patient's age range was between 15-65 years with a mean age of 48.7 years (median 49 yrs.). Peak incidence was seen in 5th decade (43%) as shown in Figure 1.

Gender Distribution

In the present study, there was slight female predominance as shown in Figure 2.

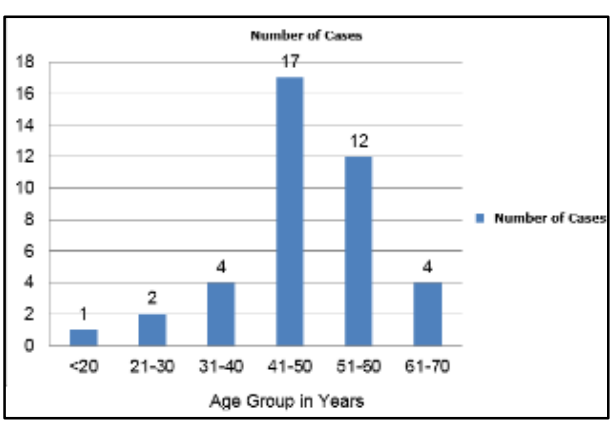

Figure 1. Age Distribution Pattern

Gender Distribution Pattern

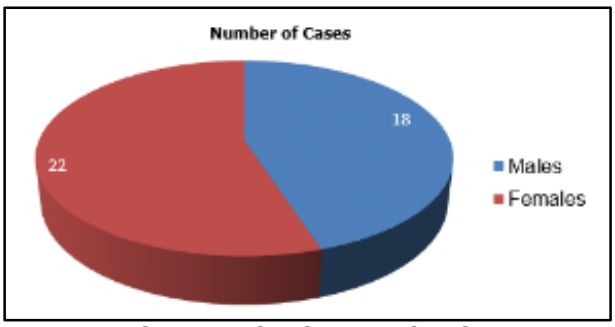

Figure 2. Pie Diagram Showing Slight Female Predominance

Clinical Features- Most common clinical presentation was pain abdomen. Acute abdomen prompting emergency surgery included intestinal obstruction, intussusceptions and appendicitis like pain. Some of the patients presented with GI bleed and mass or lump in abdomen, which is of insidious in onset and associated with weight loss.

Symptoms	Percentage of Patients
Pain abdomen	57%
Mass per abdomen/lump	35%
Bleeding	25%
Acute abdomen	20%

Table 2. Percentage of Presenting Symptoms of GIST in the Study

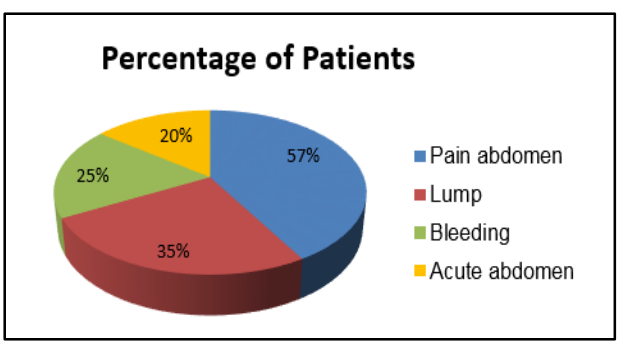

Figure 3. Pie Diagram Showing Percentage of Presenting Symptoms in GIST

Site Distribution

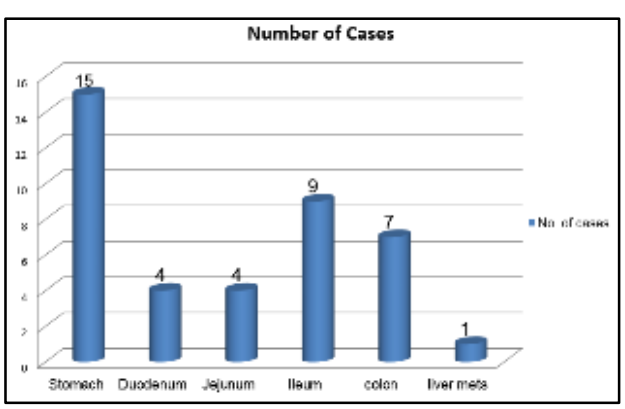

Figure 4. Bar Diagram showing Small Intestine as Common Site

Site of Origin	Number (Percentage)
Stomach (15 cases)	15 (37.5%)
Small intestine (17 cases)	
Duodenum	4 (10%)
Jejunum	4 (10%)
Ileum	9 (22.5%)
Large intestine (7 cases)	(17.5%)
Liver mets	1 (2.5%)

Table 3. Distribution of Cases Based on Site

Macroscopic Examination- The median tumour size was 10 cm (range 2.5-20 cm). Majority of tumours were of large size. Majority of these tumours presented as solid, well-circumscribed and firm mass or pedunculated, externally attached exophytic mass (Figure 8).

On cut section, these tumours are pink-tan or grey-white, granular, rubbery and firm in consistency with myxoid areas, necrosis and haemorrhage (Figure 9).

Histopathological Examination- Tumours showed variable cellularity. Majority of tumours were of spindle cell morphology followed by mixed and epithelioid pattern as shown in Table 6 and Figure 10 (a-c).

Histopathologic Patterns of Gastric GISTs- Grossly, larger GISTs presented as well circumscribed, pedunculated masses, externally attached to outer aspect of gut wall and the smaller GISTs as intramural or intraluminal polypoid mass.

On histopathological examination, 14 cases (93%) showed predominantly spindle cell pattern, 1 case (7%) showed mixed pattern consisting of both spindled and epithelioid cells.

Mitotic count of <5/50 HPF were seen in 13 cases (87%) and the remaining 2 cases (13%) showed mitotic count of >5/50 HPF.

Based on tumour size and mitotic count, majority of these tumours were of low-risk category in 6 cases (40%) and 3 cases (20%) were of moderate risk, 4 cases (27%) in very low and 2 cases (13%) in high-risk category.

3 cases (14%) showed predominantly liquefactive necrosis, coagulative necrosis is seen in only case, fibromyxoid stroma is seen in 4 cases.

On immunohistochemistry, 86% (13 cases) of cases showed CD117 positivity. Majority of cases showed strong, diffuse cytoplasmic positivity and focal cytoplasmic and membranous positivity was seen in each case. 2 cases were CD117 negative. Of these, 1 case was SMA positive, other was pan CK positive. DOG1 was positive in 86% (13 cases) of GIST, which were also CD117 positive. All of them showed strong cytoplasmic positivity for DOG1. Ki67 index was low in 13 cases (86%) and high in 2 cases (14%).

Histopathologic Patterns of Small Intestine GISTs- Grossly, majority of these tumours presented as solid, well-circumscribed and firm mass or pedunculated, exophytic mass. Few cases were seen as intramural mass and intraluminal polyp obstructing the lumen. The size of the tumours ranged 5-24 cm with a mean size of 12.6 cm,

median being 11 cms. One case presented with history of multiple neurofibromas, ovarian mass (serous cystadenoma), acute appendicitis, spindle cell tumour on bowel serosa.

Majority of small intestinal GISTs, 14 cases (82%) were of spindle cell morphology composed of spindle cells arranged in interlacing fascicles and bundles and the remaining 3 cases (18%) were of mixed cell pattern consisting of both spindle and epithelioid cells. 2 cases (12%) showed microscopically distinct, round, oval or elongated oeosinophilic and periodic acid-Schiff positive aggregates of extracellular collagen fibers known as skeinoid fibers (Figure 11). 4 cases showed mitotic count >5/50 HPF and the remaining 13 cases showed a mitotic count <5/50 HPF.

The GISTs arising from the jejunum were of spindle cell morphology. One case arising from jejunum was recurrent GIST with a mitotic activity of >5/50 HPF. Coagulative necrosis was seen in 2 cases.

Of the 9 cases arising from the ileum, majority were of spindle cell morphology, but 2 cases were of mixed cell pattern. 2 cases presented with a mitotic activity of >5/50 HPF. Coagulative necrosis was seen in 2 cases.

Based on tumour size and mitotic activity, majority of tumours were of high-risk category, 8 cases (47%), 4 cases (23%) belong to low-risk category and 5 cases (30%) belong to moderate risk category according to NIH risk categorisation.[5]

29% (5 cases) from small intestine showed coagulative necrosis. Areas of haemorrhage were seen in 2 cases. Of the total 17 cases, 2 cases showed marked nuclear pleomorphism, 4 cases with moderate and 11 cases with mild nuclear pleomorphism. Mucosal ulceration was seen in 2 cases.

On immunohistochemistry, nearly all cases showed CD117 positivity except 1 case of duodenal GIST, which was DOG1 positive. In majority of cases (94%), CD117 showed strong diffuse cytoplasmic positivity. Membranous positivity was seen in epithelioid cells of the tumours with mixed pattern. DOG1 was positive in all cases (100%) of small intestine and showed strong diffuse cytoplasmic positivity. Ki67 index showed nuclear positivity and was low in 13 cases (76%) and high in 4 cases (24%).

Histopathologic Patterns of Large Intestine GISTs- GISTs arising from large intestine showed predominantly spindle cell pattern in 5 cases (71%). 1 case showed mixed pattern with both spindle and epithelioid cells with mitotic count <5/50 hpf. One case showed epithelioid pattern with high mitotic count >5/50 hpf.

On immunohistochemistry, CD117 and DOG1 showed strong cytoplasmic positivity in majority of cases. CD117 was positive in 3 cases (43%), DOG1 was positive in 5 cases (71.4%), 2 cases (28.5%) were negative for both CD117 and DOG1 and Ki67 index was low in 5 cases (71%) and high in 2 cases (29%).

Based on tumour size and mitotic count, majority of tumours were of moderate-risk category in 5 cases (71%) and high-risk category in 2 cases (29%). Ki67 index was low in 6 cases and high in 2 cases.

Necrosis was seen in 20% of gastric GIST and 29% of small intestine and large intestinal tumours. Mucosal ulceration was present in 5 cases and was common with mitotically active tumours. Fibromyxoid stroma was seen in 2 cases. Nuclear palisading was commonly seen in 16 cases. Notable paranuclear vacuolisation was recorded in 9 cases. Skeinoid fibers were present in 2 cases arising from small intestine. Areas of calcification were noted in 2 cases. One interesting case of metastatic GIST in liver was noticed in 63 yrs. male patient who presented with multiple lesions in liver, largest measuring 6 x 5 x 3 cms as shown in Figure 12. This case showed mixed pattern histologically and on IHC CD117 and DOG1 positivity was seen with low Ki67 labelling index.

Morphological Pattern	No. of Cases	Percentage
Spindled	32	80%
Epithelioid	1	2.5%
Mixed	7	17.5%

Table 4. Distribution of Tumour Based on Morphology

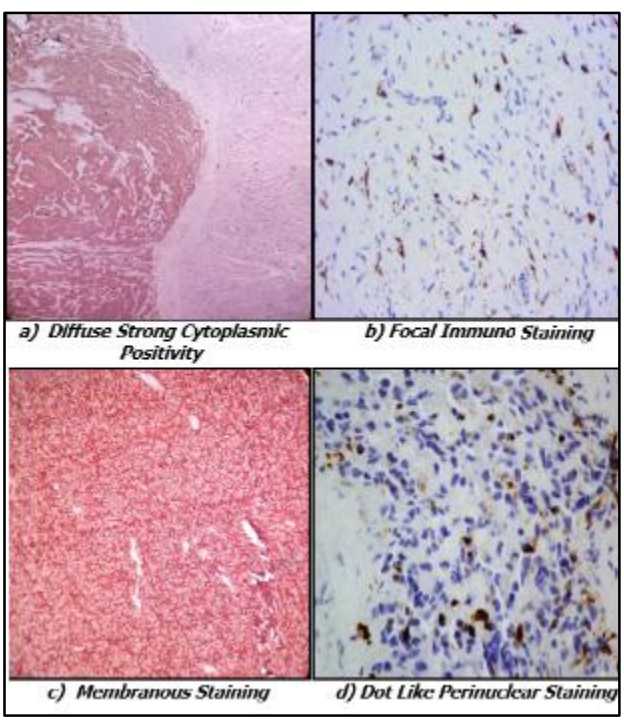

Figure 5. Immunostaining Patterns of CD117 in GIST

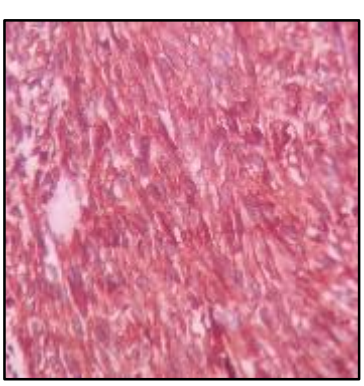

Figure 6. Immunostaining Pattern of DOG1 in GIST Showing Diffuse Cytoplasmic Positivity

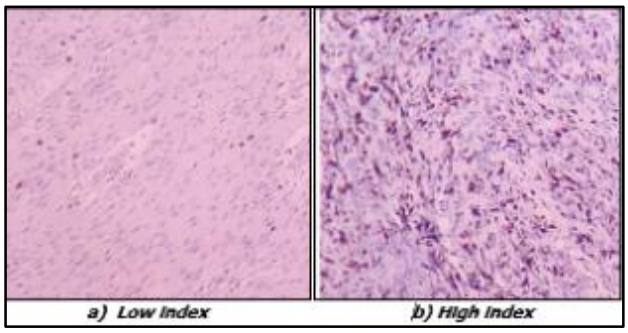

Figure 7. Ki67 Index Showing Nuclear Positivity

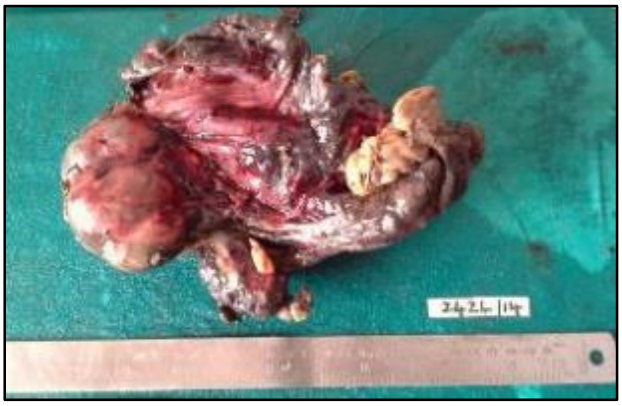

Figure 8. Gross Photograph of Ileal GIST m/s 20 x 15 x 10 cms. Grey brown in Colour, Unencapsulated, Surface is Nodular, Irregular and Exophytic

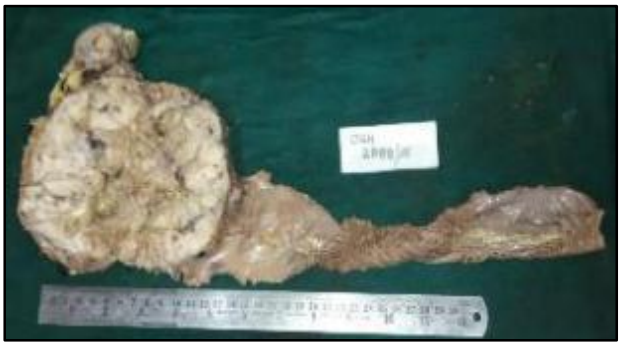

Figure 9. Cut Section of Duodenal GIST, Well-Circumscribed, Grey White in Colour

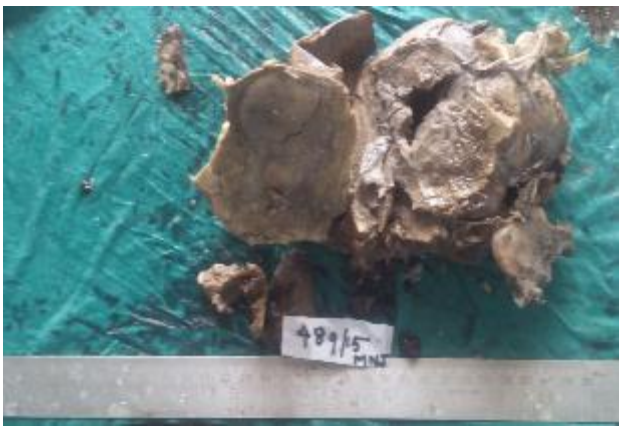

Figure 10. Gross Photograph of GIST Metastasis to Liver

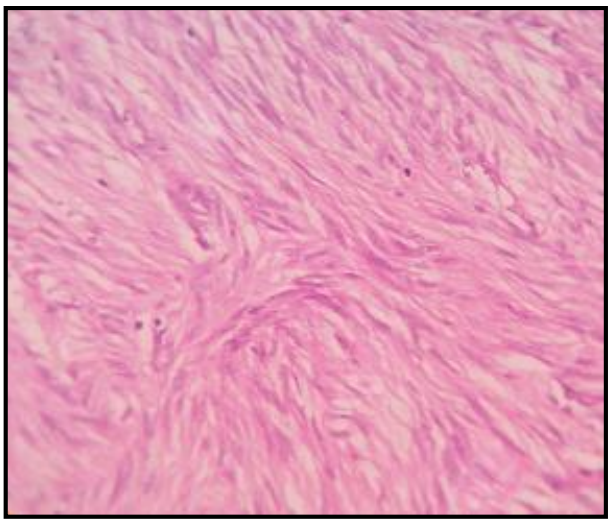

Figure 10a. Microphotograph of Spindle Cell Pattern (40x)

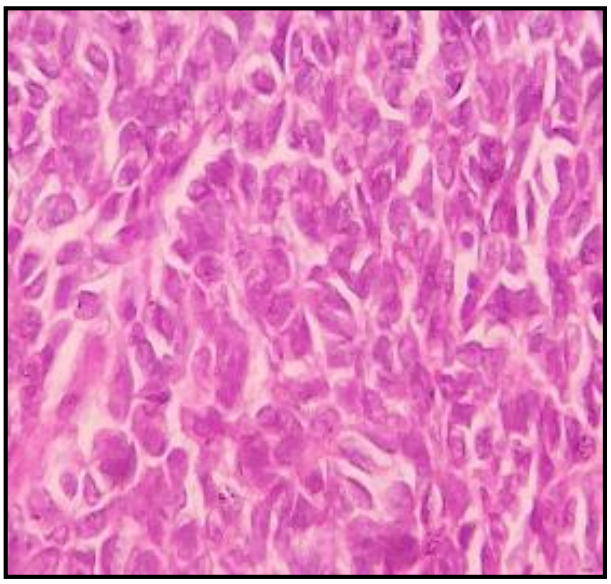

Figure 10b. Microphotograph of Mixed Variant (40x)

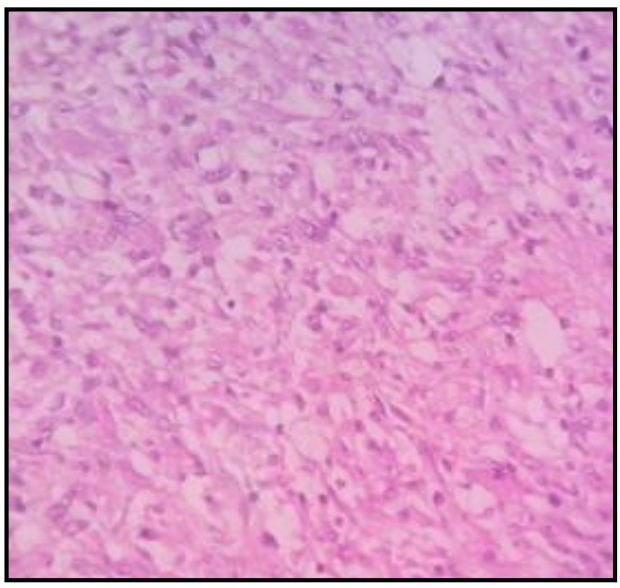

Figure 10c. Microphotograph of Epithelioid Variant (40x)

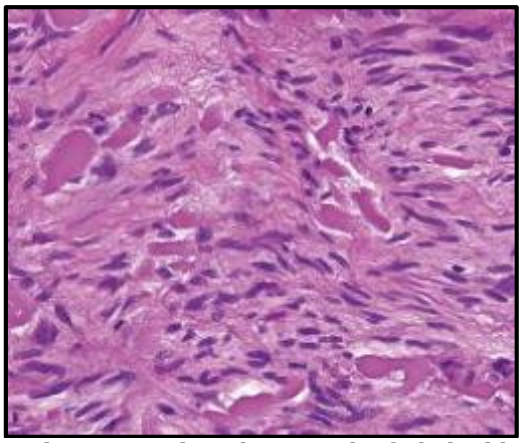

Figure 11. Microphotograph of Skeinoid Fibres in Intestinal GIST (40x)

DISCUSSION

GIST shows wide morphologic spectrum.[6]
Spindle cell - Sclerosing, palisading vacuolated, hypercellular and sarcomatous variants.
Epithelioid- Sclerosing, discohesive, hypercellular and sarcomatoid.

A variety of mesenchymal tumours should be considered in the differential diagnosis of GIST. Immunohistochemical studies that include CD117 have become valuable in the classification of mesenchymal lesions arising in the gastrointestinal tract. C-Kit and DOG1 expression by immunohistochemistry is considered as a key diagnostic feature in GISTs.

In the present study, 9 cases (22.5%) were negative for CD117. The 2 cases (5%) showed histopathological features of GIST and were negative for CD117, but IHC with DOG1 antibody showed diffuse strong cytoplasmic positivity, which is probably attributed to mutations in Platelet-Derived Growth Factor Alpha (PDGFRA).

PDGFRA mutations show a strong predilection to gastric GISTs with epithelioid morphology. However, a few non-gastric PDGFRA mutant, GISTs have also been reported, especially in the duodenum. Initially, PDGFRA mutations were reported in 35% of KIT mutation negative GISTs.[7]

DOG1 has shown a high overall sensitivity and specificity for the detection of GISTs. Furthermore, DOG1 immunoreactivity is rarely observed in other mesenchymal and non-mesenchymal tumour types. These results support the use of DOG1 as a good diagnostic biomarker for GIST.[8]

Even when both CD117 and DOG1 are negative, it is legitimate to make the diagnosis of GIST on morphologic grounds, but this is a very strong indication to refer paraffin blocks to a center capable of mutational analysis.[9]

CONCLUSION

- In the present study, we studied a total of 40 cases with a mean age of 48 years and the peak age incidence in 5th decade.
- GISTs are more prevalent in small intestine followed by stomach and large intestine.
- Most common clinical presentation was pain abdomen seen in 64% of cases followed by GI bleed in 56% of cases.
- Majority of the tumours were of large size with a varied range of 2.5-20 cm. A total 12 cases (30%) presented with a size >10 cm, 19 cases (47.5%) between the size range of 5-10 cm and 8 cases (22.5%) within size range of 2-5 cm.
- On histopathological examination, 80% of GISTs showed spindle cell morphology, 17.5% showed mixed pattern and 2.5% showed epithelioid pattern.
- Based on tumour site, size and mitotic count, 3 (7.5%), 12 (30%), 13 (32.5%), 12 (30%) of the patients were classified into very low, low, intermediate and high-risk groups respectively according to the NIH risk categorisation.
- On immunohistochemistry, CD117 (77.5%), DOG1 (85%) positivity.
- Ki67 over expression is significantly correlated with larger GISTs.

REFERENCES

[1] Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumours. N Engl J Med 2002;347(7):472-480.
[2] Lee CH, Liang CW, Espinosa I. The utility of discovered on gastrointestinal stromal tumour 1 (DOG1) antibody in surgical pathology-the GIST of it. Adv Anat Pathol 2010;17(3):222-232.
[3] Zhao WY, jia XU, Wang M, et al. Prognostic value of Ki67 index in gastrointestinal stromal tumours. Int J Clin Exp Path 2014;7(5):2298-2304.
[4] Yamaguchi U, Hasegawa T, Masuda T, et al. Differential diagnosis of gastrointestinal stromal tumour and other spindle cell tumours in the gastrointestinal tract based on immunohistochemical analysis. Virchows Arch 2004;445(2):142-150.
[5] Fletcher CD, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumours: a consensus approach. Hum Pathol 2002;33(5):459-465.
[6] Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumours of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol 2005;29(1):52-68.
[7] Rubin BP, Singer S, Tsao C, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumours. Cancer Res 2001;61(22):8118-8121.
[8] West RB, Corless CL, Chen X, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumours irrespective of KIT or PDGFRA mutation status. Am J Pathol 2004;165(1):107-113.
[9] Liegl B, Hornick JL, Corless CL, et al. Monoclonal antibody DOG1.1 shows higher sensitivity than KIT in the diagnosis of gastrointestinal stromal tumours including unusual subtypes. Am J Surg Pathol 2009;33(3):437-446.