Pathological Spectrum of Gastrointestinal Stromal Tumors - A 1.5-year Experience at Kidwai Cancer Institute

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors arising in the gastrointestinal tract. Most GISTs were thought to be of smooth muscle origin but have now been found to be distinctive entities based on morphological, immunohistochemical, and ultrastructural studies.¹ GISTs are thought to arise from interstitial cells of Cajal both of which show KIT positivity.²

According to most studies, GISTs have an incidence of 10–15 per million per year with a median age of presentation in the mid-60s with equal sex distribution. GISTs commonly involve the stomach followed by the small intestine. Other sites of involvement include colorectum, rarely esophagus, and appendix.³ GISTs can also be found in the omentum, mesentery, or the retroperitoneum and are referred to as extra-gastrointestinal tract tumors (EGISTs).⁴

The pathogenesis of sporadic GISTs is thought to be driven by Gain-of-function mutations in the genes encoding tyrosine kinase receptors KIT and PDGFRA, both of which are located on chromosome 4q.⁵ Rarely,
GISTs can show loss of succinate dehydrogenase expression. They typically lack KIT or PDGFRA mutations, and the majority of them are seen in pediatric age group, and some may be part of Carney’s triad.[6] GISTs have also been discovered in patients with neurofibromatosis type 1.[4]

Morphologically, GISTs are composed of spindle cells in the majority of the cases, epithelioid cells in few cases and rarely a mixed morphology. Immunohistochemically majority of GISTs show KIT positivity with a minority of cases being KIT-negative. Other markers which could be positive include CD34, SMA, and rarely S100 but are mostly negative for desmin.[10] Discovered on GIST 1 (DOG1) is a recently discovered sensitive marker for GISTs which also stains a subset of KIT-negative cases.[8,9] Mitotic rate and tumor size have been found to be of predictive value in the behavior of GISTs. These parameters have been used to stratify GISTs into low, intermediate, and high-risk categories to predict the clinical behavior of these tumors.[8,9] The treatment of GISTs includes surgery and imatinib therapy. Imatinib is a selective inhibitor of tyrosine kinase receptors especially KIT and PDGFRA which is especially useful in advanced GISTs and high-risk GISTs.[11]

**MATERIALS AND METHODS**

This hospital-based study was conducted at Kidwai Cancer Institute, Bengaluru. This prospective study included patients diagnosed with GISTs in our institute as well as those cases referred from other hospitals over a period of 1½ years from January 2016 to June 2017. The study protocol was approved by the Institutional Review Board and the Ethical Committee.

**Informed Consent**

Patients who voluntarily registered at the hospital were taken for the study for which a panel of immunohistochemical markers, which are done on a routine basis to confirm a diagnosis of GIST, were performed. Informed consent was taken from the patients selected for next-generation sequencing (NGS). Both immunohistochemistry (IHC) and NGS were performed on formalin-fixed paraffin-embedded (FFPE) tissue blocks from the material sent by the clinician for diagnostic purpose.

**Patient Selection**

This study was conducted on patients with pathologically confirmed GISTs in the Department of Pathology, Kidwai Cancer Institute, Bengaluru, over a period of 1½ years from January 2016 to June 2017. Furthermore, other stromal tumors of the gastrointestinal tract other than GISTs were studied for comparison.

**Clinical Data**

Patient’s clinical details including age, gender, site of involvement, and treatment history were collected from medical records of the patients.

**Sample Collection**

FFPE tissue blocks including cell blocks made from the material (which included biopsies, resection specimens, and FNA material) sent by the clinician and FFPE tissue blocks received from other institutions were collected, and H and E, as well as IHC slides, were prepared from the same.

**Inclusion Criteria**

1. Patients with pathologically confirmed GIST whose paraffin-embedded tissue blocks were available at Kidwai Cancer Institute, Bengaluru.
2. KIT-negative GISTs which were diagnosed on morphology.
3. Patients with other mesenchymal tumors other than GISTs arising from the gastrointestinal tract, retroperitoneum and presenting as intra-abdominal masses, including tumors of myogenic, neurogenic, and fibroblastic origin were studied for comparison.

**Exclusion Criteria**

1. Patients with GISTs whose paraffin-embedded tissue blocks were unavailable at Kidwai Cancer Institute, Bengaluru.
2. Patients with other mesenchymal tumors other than GISTs arising from the gastrointestinal tract, retroperitoneum and presenting as intra-abdominal masses whose paraffin-embedded tissue blocks were unavailable at Kidwai Cancer Institute, Bengaluru.
3. Biopsies which lacked adequate material to perform IHC were excluded from the study.

**IHC (n = 52)**

A one-step polymer-horseradish peroxidase detection system was used.

**Procedure**

Tissue sectioned (3 µ) on poly-L-Lysine slides was deparaffinized, treated with an antigen retrieval solution, blocked with a peroxidase 2% skimmed milk blocking solution and then incubated with the primary antibody. The primary antibody binds to the antigen of interest. This was followed by incubation with the secondary antibody conjugated with horseradish peroxidase polymer and color development using 3,3’-diaminobenzidine substrate. When adequate color development was seen, the slides were washed in water to stop the reaction, counterstained with hematoxylin and covered with a mounting medium.

**Next-generation Sequencing (n = 3)**

The cases which were C-kit-negative and DOG1-positive were analyzed for mutational status using NGS to look
for KIT and PDGFRA mutations or any other mutations present.

Genomic DNA was extracted from 10 µm sections obtained from the FFPE blocks. QIA amp DNA FFPE Tissue Kit was used for DNA extraction. Quantitation and QC of extracted DNA were carried out using Nanodrop, Bioanalyzer/Tapestation.

Procedure
Illumina sequencing by synthesis (SBS) chemistry was used for our cases, and it included 4 basic steps:

1. Library Preparation: Libraries were prepared using 250 ng of the genomic DNA. The TruSeq Amplicon Cancer Panel Kit paired with the TruSeq Amplicon Cancer Panel (212 DNA-specific amplicon covering hotspots in 48 genes) was used to construct the libraries. The sequencing library was prepared by random fragmentation of the DNA sample, followed by 5’ and 3’ adapter ligation. Adapter-ligated fragments were then PCR amplified and gel purified.

2. Cluster generation: For cluster generation, the library was loaded into a flow cell where fragments were captured on a lawn of surface-bound oligonucleotides complementary to the library adapters. Each fragment was then amplified into distinct, clonal clusters through bridge amplification.

3. Sequencing: Illumina SBS technology utilizes a proprietary reversible terminator-based method that detects single bases as they are incorporated into DNA template strands.

4. Data analysis: During data analysis and alignment, the newly identified sequence reads were then aligned to a reference genome.

Statistical Analysis
The collected data were subjected to statistical analysis using SPSS version 21 and STATA version 13 software. Frequency tables were generated on the parameters studied. Sensitivity and specificity of KIT and DOG1 were calculated based on receiver operating characteristic curve, and Chi-square test was used to correlate Ki67 with the risk of metastasis.

RESULTS
There were 39 cases of mesenchymal tumors of the GIT after excluding tumors from sites outside the GIT such as retroperitoneum and omentum. Of these cases, there were 32 cases of GISTs (82%), 3 cases of leiomyosarcomas (8%), 2 cases of leiomyoma (5%), 1 case of schwannoma (3%), and 1 case of solitary fibrous tumor (3%).

Of 37 cases of GIST (including EGIST), there were 22 males and 15 females with a male to female ratio equal to 1.5. The age of presentation of GIST ranged from 22 to 72 years with a mean of 52.8 years. The mean age for males was 53.6 years while the mean age for females was 51.6 years in this study. No case of pediatric GIST was encountered in this study. The most common presenting symptom of GIST was pain abdomen which was seen in 17 cases (46%) followed by mass per abdomen in 5 cases (14%). There were 14 cases of GISTs involving the stomach (38%) followed by 12 cases in the small intestine (32%) [Figure 1]. Other sites of involvement included 2 duodenal GISTs, 1 colonic GIST, and 3 rectal GISTs. There were 5 cases of EGISTs which included retroperitoneal, omental, and pelvic GISTs. The size of GISTs ranged from 1.5 to 29 cm with a mean of 10.7 cm. The size distribution of GISTs was as follows - 1 case with size <2 cm (3%), 3 cases with size 2–5 cm (8%), 16 cases with size 5–10 cm (43%), and 17 cases with size >10 cm (46%) [Figure 2]. Of the 37 cases of GISTs, 28 cases had a spindle cell morphology (76%), 6 cases had an epithelioid morphology (16%), and 3 cases had a mixed spindle and epithelioid morphology (8%) [Figures 3–5]. Necrosis was seen in 9 cases (24%) of GIST, all cases had spindle cell morphology, and 77.8% of cases belonged to the high-risk category.

Expression of Kit and Dog1 in GISTs
In this study, kit was positive in 91.9% of cases, and DOG1 was also positive in 91.9% of cases [Figures 6 and 7]. Of 37 cases of GIST, 32 cases showed both KIT and DOG1 positivity (82%), 2 cases showed only KIT positivity (8%), 2 cases showed only DOG1 positivity (8%), and 1 case was both kit- and DOG1-negative (3%) [Figure 8]. Both kit and DOG1 when used together identified 36 of 37 cases of GIST (97.3%). IHC for CD34 was available in 30 cases of GISTs, and 18 cases were positive (60%).

Figure 1: Distribution of gastrointestinal stromal tumors cases by site
IHC for SMA was available in 30 cases of GISTs and 21 cases were positive (70%). IHC for S100 was available
in 27 cases of GISTs, and 1 case was positive (3.7%). IHC for desmin was available in 25 cases of GISTs and 2 cases were positive (8%).

**Mutational Analysis by NGS**

There were 3 cases which were KIT-negative and DOG1-positive on IHC with CD34, SMA, S100, and desmin being negative. Mutational analysis was done for these 3 cases of which 2 showed mutations consistent with GIST. The remaining one case was negative for both KIT and PDGFRA and was diagnosed with biphasic malignant mesothelioma after additional IHC markers such as WT1 and D2-40 showed positivity. One case of KIT IHC negative GIST, involving the stomach had KIT point mutations involving exon 17. Other mutations present included NRAS, IDH1, and TP53. The other case involving the retroperitoneum harbored PDGFRA point mutations involving exon 18. Other mutations present included NRAS [Table 1].

Treatment details were available only for 14 of the 37 cases of GIST. 5 cases underwent only surgical resection (36%), 3 cases received only imatinib therapy (21%), 5 cases had both surgical resection and imatinib therapy (36%), and one case did not receive any treatment. One of the 3 cases which received only imatinib therapy showed resistance to imatinib and was started on sunitinib.

**Prognosis**

Of the 37 cases, there were 32 cases of primary GISTs of which 6 cases presented with metastatic disease. Remaining 5 cases were recurrent GISTs. Statistical analysis showed an association between epithelioid and mixed morphology and a high rate of recurrence and metastatic disease [Table 2]. Fletcher’s criteria were used to prognosticate risk of metastasis in 31 cases of GISTs without metastasis [Table 3]. There were 26 cases (83.9%) in the high-risk category, and 5 cases (16.1%) were in the intermediate risk category. There were no cases in the low and very low-risk categories. Site wise prognostication for risk of metastasis was used to categorize GISTs of different sites into 8 groups (1, 2, 3a, 3b, 4, 5, 6a, and 6b) [Table 4].

In this study, all GISTs were encompassed in one of four categories, which were the groups 3a, 3b, 6a, and 6b. There were no cases in the remaining categories. After site wise categorization of the cases 83% gastric GISTs, 67% of small intestinal GISTs, and 100% each of duodenal and rectal GISTs were in the high-risk group while 17% of gastric GISTs and 33% of small intestinal GISTs were in the medium risk group. There were no cases in the low-risk group. The Ki67 index ranged from 3 to 45%. We found an association between Fletcher’s prognostic criteria and Ki67 index for risk of metastasis when the cutoff for Ki67 was taken at 8% [Table 5].

**DISCUSSION**

GISTs are the most common mesenchymal tumors of the gastrointestinal tract. The discovery of KIT mutations in GISTs and the available targeted therapy, the tyrosine kinase inhibitor imatinib, has necessitated the need for an accurate diagnosis of GISTs. In this study, 82% of true mesenchymal tumors of the GIT were GISTs followed by leiomyosarcoma and leiomyoma. Rarely, cases of schwannoma and solitary fibrous tumor were diagnosed. This was comparable with other studies by Yamaguchi et al. and Patnayak et al. The mean age of presentation in this study was 52.8 years with females presenting at a
slightly earlier age than males. This was comparable with Indian data obtained from studies by Patnayak et al.,[14] Ravikumar et al.,[15] and Lakshmaiah et al.[16] whereas global data had a higher median age of presentation of around 60 years.[3] A slight male predominance was observed in this study with a male to female ratio of 1.5:1 which was comparable with other Indian studies by Patnayak et al.[14] and Rajappa et al.[17] Global data showed equal distribution between males and females.[19]

GISTs have a varied clinical presentation with pain abdomen, GI bleeding, intestinal obstruction, and mass per abdomen being the most common symptoms encountered in both Indian studies and global studies.[13,15-17] Pain abdomen followed by mass per abdomen was the most common symptoms encountered in this study. Patients presented with localized GIST in 26 cases (70.3%), metastatic GIST in 6 cases (16.2%), and recurrent GIST in 5 cases (13.5%). This was similar to studies by Sharma.[18] A study by Bhalgami et al.[19] had a higher incidence of presentation with metastatic GIST. The sites of metastasis in this study included liver, omentum, abdominal wall, and lung. Sites of recurrence included abdominal wall, lower abdominal cavity, and a rare case of spermatic cord recurrence in a treated case of small intestinal GIST. Stomach was the most common site of involvement followed by small intestine in this study which was similar to other Indian and global studies.[3,14,17] Tumor size varied from 1.5 to 29 cm with a mean size of 10.7 cm. Majority of GISTs had a size of more than 10 cm (46%). This was similar to data obtained from both Indian studies and global epidemiological data.[3,14,15,17] Spindle cell pattern was the most common histological pattern observed in this study and was present in 28 cases (76%) which was in concordance with studies by Vij et al.,[20] Kim et al.,[21] and Lakshmi et al.[22] Epithelioid pattern was observed in 6 cases (16%) and mixed epithelioid and spindle cell pattern was observed in 3 cases (8%) which in comparison to other studies showed slightly more cases of epithelioid morphology than mixed morphology.[20-22] Epithelioid morphology was more commonly seen in EGISTs and small intestinal GISTs accounting for 2 cases each. This study showed that epithelioid and mixed morphology have a higher rate of recurrence and metastatic disease than spindle cell morphology. Secondary changes such as necrosis, hyalinization, and calcification were also noted in GISTs. Necrosis was noted in 9 cases (24%) out of which 7 cases (77.8%) belonged to high-risk category, similar to a study by Kim et al.[21] IHC is a highly sensitive tool in the diagnosis of GISTs. KIT and recently DOG1 have primarily emerged as diagnostic markers of GIST. KIT positivity on IHC varied from focal–to-diffuse and weak–to-strong cytoplasmic and membranous positivity. In this study, KIT showed a sensitivity of 91.9% and a specificity of 100% [Table 6]. Few studies have shown a slightly higher rate of positivity for KIT while one Indian study has shown a lower positivity rate for KIT [Table 7]. DOG1 positivity on IHC varied from focal to diffuse and weak to strong cytoplasmic and membranous positivity. DOG1 showed a sensitivity of 91.9% and a specificity of 76.9% in this study. Different studies show varying positivity rates for DOG1.

### Table 2: Correlation between morphology and recurrence or metastasis

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Spindle (%)</th>
<th>Epithelioid (%)</th>
<th>Mixed (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>22 (78.6)</td>
<td>3 (50)</td>
<td>1 (33.3)</td>
<td>26 (70.3)</td>
</tr>
<tr>
<td>Recurrent</td>
<td>2 (7.1)</td>
<td>1 (16.7)</td>
<td>2 (66.7)</td>
<td>5 (13.5)</td>
</tr>
<tr>
<td>Metastatic</td>
<td>4 (14.3)</td>
<td>2 (33.3)</td>
<td>0 (0)</td>
<td>6 (16.2)</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>6</td>
<td>3</td>
<td>37</td>
</tr>
</tbody>
</table>

**P < 0.05**

### Table 3: Approach for defining risk of aggressive behavior in GISTs - Fletcher’s criteria

<table>
<thead>
<tr>
<th>Risk</th>
<th>Size (single largest dimension) (cm)</th>
<th>Mitotic count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low-risk</td>
<td>&lt;2</td>
<td>&lt;5/50 HPF</td>
</tr>
<tr>
<td>Low-risk</td>
<td>2–5</td>
<td>&lt;5/50 HPF</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&lt;5</td>
<td>6–10/50 HPF</td>
</tr>
<tr>
<td>High-risk</td>
<td>&gt;5</td>
<td>&gt;5/50 HPF</td>
</tr>
<tr>
<td></td>
<td>&gt;10</td>
<td>Any mitotic rate</td>
</tr>
<tr>
<td></td>
<td>Any size</td>
<td>&gt;10/50 HPF</td>
</tr>
</tbody>
</table>

### Table 4: Prognostication of GISTs of different sites

<table>
<thead>
<tr>
<th>Tumor parameters</th>
<th>Percentage of patients with progressive disease during long-term follow-up and characterization of risk for metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Gastric GISTs (%)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.9 (VL)</td>
</tr>
<tr>
<td>3a</td>
<td>3.6 (L)</td>
</tr>
<tr>
<td>3b</td>
<td>12 (M)</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>16 (M)</td>
</tr>
<tr>
<td>6a</td>
<td>55 (H)</td>
</tr>
<tr>
<td>6b</td>
<td>86 (H)</td>
</tr>
</tbody>
</table>

GIST: Gastrointestinal stromal tumors
The positivity rates observed in this study was comparable with a study by Xu et al. [Table 8].

When using IHC for KIT and DOG1 in combination to identify GIST, the sensitivity increased to 97.3% which was comparable with the study by Xu et al. while the specificity was 76.9%. This indicated the usefulness of combining the two markers in identifying cases of GIST. Other markers which were positive in a subset of GISTs include CD34, SMA, S100, and desmin. CD34 was positive in 60% of cases which was similar to studies by Güler et al. Bhalgami et al. and Vij et al. SMA was positive in 70% of cases which was higher than studies by Güler et al., Kim et al., and Vij et al. S100 was positive in 3.7% of cases which was comparable to the study by Güler et al. Desmin was positive in 8% of GISTs in this study which was lower than studies by Bhalgami et al. and Ueyama et al.

This study encountered 3 cases which showed KIT negativity and DOG1 positivity on IHC. Mutational analysis was performed for these three cases to look for KIT, PDGFRA, or other GIST associated mutations. One case of KIT-negative GIST harbored PDGFRA D842V point mutation involving exon 18 which is the most common PDGFRA mutation reported in literature. The site of involvement was retroperitoneum. This case had an epithelioid morphology which is commonly seen in PDGFRA exon 18 mutations. The second case harboring KIT exon 17 mutations, showed a spindle cell morphology and was located in the stomach. The patient presented with lung metastasis and died within 2 months. The patient did not receive any treatment. KIT exon 17 mutations are rare KIT mutations seen in <1% of GISTs. Interestingly both these cases showed an NRAS mutation (Q61K) in addition to KIT or PDGFRA mutations, respectively. RAS family mutations can rarely occur in GISTs and account for <1% of all GISTs.

In comparison to our study, other studies showed a slightly lesser number of cases in the high-risk category while there was the similar distribution of cases in the intermediate risk category [Table 9].

A study by Zhao et al. suggested that a cutoff value of 8% for Ki67 can effectively sub-divide high-risk patients of GIST who was substantiated in this study. Therefore, Ki67 can be used as an adverse prognostic marker in GIST.

CONCLUSION

The following conclusions were drawn from this study:

- Out of all the true mesenchymal tumors of the GIT, the gastrointestinal stromal tumor was the most common.
- The age of presentation was earlier in this study as well as Indian studies when compared to global data. This study showed a slight male predominance similar to other Indian studies whereas global data showed equal sex distribution.
- GISTs commonly present with pain abdomen. Metastatic disease was present in 16.2% of cases while...
the recurrence of GISTs was seen in 13.5% of cases.
- Stomach was the most common site of involvement, and the mean tumor size was 10.7 cms.
- Spindle cell morphology was more commonly present while epithelioid and mixed morphology carried a higher recurrence rate.
- Although KIT is still the most specific marker for GIST, the combination of KIT and DOG1 highly improved the sensitivity of identifying cases of GIST.
- Mutation analysis in KIT-negative cases can be helpful in identifying KIT, PDGFRA, and other associated mutations in GIST.
- Ki67 can be used as an adverse prognostic marker for identifying high-risk cases of GIST.

REFERENCES