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Original Article

Role of Novel Marker Discovered on Gastrointestinal Stromal Tumor 1 in Evaluation of Gastrointestinal Stromal Tumors

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Abstract

Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors arising from myenteric ganglion cells, termed interstitial cells of Cajal. GISTs occur predominantly between 40 and 60 years of age. CD34 was the initially used for diagnosing GIST. Due to its low specificity for GISTs, CD34 was replaced by C-KIT, which is a reliable marker. However, 5% GISTs lack C-KIT expression. Recent studies have shown GIST1 (DOG1) to be a more sensitive and specific marker compared to C-KIT and CD34. **Aims and Objectives:** The aim was to study histomorphology characteristics and risk stratification of all cases previously diagnosed as GISTs, to evaluate these cases for CD117 and DOG1 expression by immunohistochemistry (IHC) and to see whether there was any advantage in using novel markers (i.e. DOG1) as compared to conventional (C-KIT) in GIST at our center. **Materials and Methods:** Fifty patients with histomorphologic or imaging impression of GIST were subjected to IHC using C-KIT and DOG1. **Results and Conclusion:** Of 50 cases 47 (94%) were positive for C-KIT, and all 50 (100%) cases were positive for DOG1. Hence, DOG1 was positive even in C-KIT-negative cases. Therefore, our study suggests that DOG1 should be added to workup of suspected cases of GIST along with C-KIT.

Keywords: *Discovered on gastrointestinal stromal tumors 1, gastrointestinal stromal tumors, tumors*

Introduction

Gastrointestinal stromal tumors (GISTs) are here defined as specific, generally Kit (CD117)-positive and Kit or platelet-derived growth factor alpha (PDGFRA) mutation-driven mesenchymal tumors of the gastrointestinal (GI) tract.[1] Most of the GISTs harbor C-KIT receptor tyrosine kinase (RTK) gene mutation or homologous RTK, PDGFRA mutation.[2] The inhibition of these tyrosine kinases (TK) has revolutionized the therapy of these tumors, as specific targeted treatment with TK inhibitors is now available.[3-5] Most kit mutations in GISTs involve exon 11 (60%–70%), which is a juxtamembranous domain with regulatory function.[6,7] Less frequent mutations occur in PDGFRA.[8,9] 10% of GISTs having no detectable mutations in these two tyrosine kinases and are referred to as wild-type of GISTs.[10] Discovered on GIST1 (DOG1), a protein encoded by Anoctamin 1, also known as transmembrane protein 16A is a calcium chloride regulated channel.[11] In contrast to other markers, DOG1 antibody shows exclusive staining of tumor cells and Interstitial cells of Cajal with no background staining. However, given that between 36% and 50% of CD117-negative tumors are DOG1 positive, this antibody should be included in the routine histochemical diagnosis of GISTs.[12] Histologically, GISTs are classified into spindle cell pattern (60%–70%), epithelioid pattern (20%), and mixed (10%). Features that increase suspicion of malignancy include an extragastric tumor location, larger size, high mitotic counts, and the presence of necrosis.[13,14] In this study, immunohistochemical staining for DOG-1 and C-Kit was performed on GISTs to help determine the utility of these markers in diagnosing and differentiating them from other morphologically similar entities.

Materials and Methods

This study was conducted in the Department of Pathology Sher-i-Kashmir Institute of Medical Sciences (SKIMS) Soura, Srinagar Kashmir. It was an observational study

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conducted over a period of 7 years, carried from 2010 to 2016. Of the 90 cases with mesenchymal tumours of the GI tract diagnosed at our center, 77 were GISTs. All the patients diagnosed with GISTs were enrolled in our study and the data were reviewed and analyzed. Paraffin-embedded blocks were retrieved from histology archives and 50 cases were obtained. Prospectively, cases suspected of having GIST were evaluated clinically. These cases were followed up after surgical removal of the tumor and 27 cases were found to have GIST. The tissue specimens, fixed in 10% formalin, were then studied for the gross findings. The tissues were then processed, and paraffin embedded blocks were obtained. 5 μ sections were cut from each block and slides prepared were stained with Haematoxylin and Eosin. These slides were then reviewed to ascertain that histomorphology was compatible with the diagnosis of GIST and to establish whether there was sufficient tumor tissue on the slide. The tumors were classified into spindle cell, epithelioid or mixed depending on the predominant cell type. Based on this information, a total of 77 (including 50 retrospective and 27 prospective cases) were collected for immunohistochemistry (IHC) for CD117 and DOG1. Formalin-fixed paraffin-embedded blocks were retrieved and sectioned to 3 μm. The antibody dilution and process of staining were carried out according to instructions. Phosphate buffer saline was used as negative control to primary antibodies. DOG-1 staining was mainly localized in cytoplasm, and few cases showed membrane staining. C-KIT also showed mostly cytoplasmic, and few showed membrane positivity. Cells were categorized according to the positive rate: Negative = Number of positive cells <5%, weak positive (+) pale brown particles = Number of positive cells 5–25%, positive (++) brown particles = Number of positive cells 25–50%, and strong positive (+++) dark brown particles = Number of positive cells >50%.

Observations and Results

A total of 90 mesenchymal tumors of the GIT and pancreas diagnosed in SKIMS from January 2010 to December 2016 were reclassified on the basis of morphologic features and IHC into 77 (85.6%) cases of GIST and the remaining 13 (14.4%) cases as other mesenchymal tumors. The mesenchymal tumors included five cases of inflammatory myofibroblastic tumors, two cases of leiomyoma, four cases were sarcomas, one case was poorly differentiated carcinoma, and one was desmoid tumor. C-KIT positivity was seen in 72 (93.5%) cases and DOG 1 was positive in 77 (100%) cases [Figures 1-4]. However, the correlation between DOG1 and CKIT was found to be statistically insignificant ($P = 0.23$). There was no significant association between DOG 1 expression and various histopathological parameters in the studied cases. Clinicopathological variables have been enumerated in Table 1.

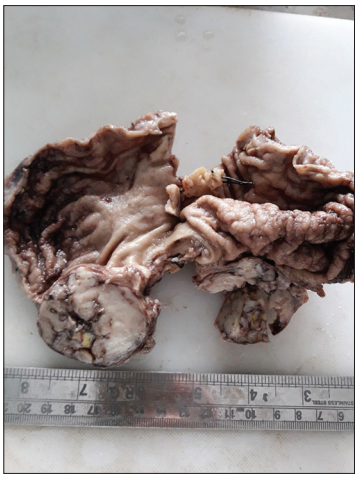

Figure 1: Gross specimen of gastrointestinal stromal tumor

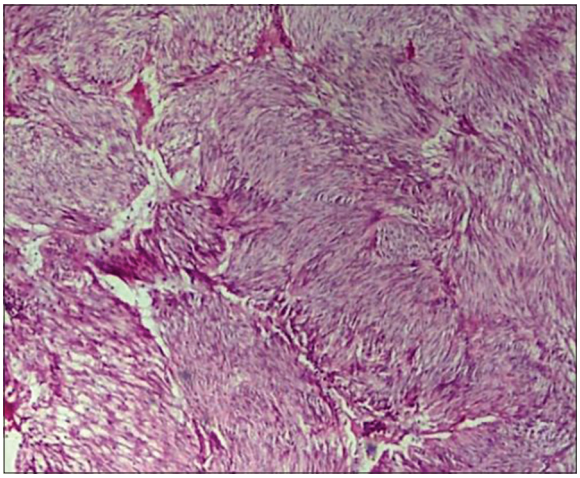

Figure 2: Microscopic view of gastrointestinal stromal tumor

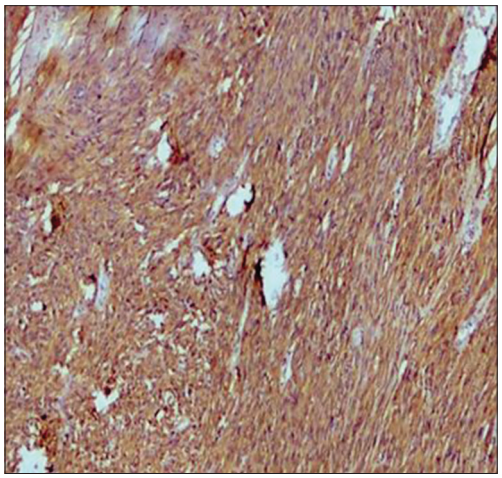

Figure 3: CKIT staining

Discussion

The diagnosis of GISTs is based on tumor location, histology, and IHC (C-KIT positivity).[15] While the combination of above characteristics identifies most of the GISTs, a fraction of GISTs remain unidentified. This

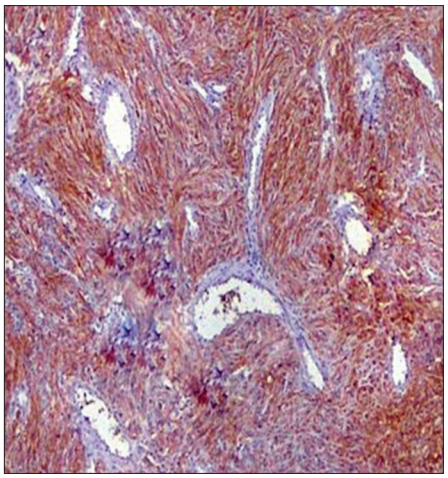

Figure 4: Discovered on gastrointestinal stromal tumor 1 staining

fraction includes the cases which are either negative or equivocal on IHC for CKIT and hence are difficult to diagnose. One way to make a diagnosis is by screening for CKIT and PDGFRA mutations, but this approach adds to time and cost; therefore, there is need to look for a marker which is accurate and adds little to time and cost. This subject has been of particular interest to histopathologists around the globe in the last decade. The aim is to identify a marker that can reliably stain CKIT negative GISTs. One such marker, which offers hope, is DOG1.[16] Our study was aimed at studying the relevance of CKIT and DOG1 on IHC in the diagnosis of GIST as well as the demographic and clinicopathologic spectrum of GISTs at our center.

Clinicopathological parameters

We studied a total of 90 patients with mesenchymal tumors of the GIT reporting at our hospital over a period of 7 years. GIST was the most common (85.6%, *n* = 77) mesenchymal GI tumor. Other mesenchymal tumors included inflammatory myofibroblastic tumors, leiomyomas, and leiomyosarcomas. This was in accordance with a study done by Lakshmi *et al*. in which, GIST constituted 52.8% of the mesenchymal tumors followed by smooth muscle cell tumors (38.1%).[17] Most of the patients in our study were in the 4th–5th decade with age ranging from 3 to 80 years. Mean age of patients was 50.7 ± 14.24 years. Similarly, in a study done in SG PGI Lucknow by Vij *et al*., the age of patients ranged from 15 to 83 years with a mean age of 50.4 years.[18] In our study, we found that GISTs are more common in males as shown in Table 1. This male predominance among GISTs was also observed in other studies by Liegl *et al*. and Gina *et al*.[3,19] In our study, GI bleeding was a common clinical presentation of GISTs as seen in some other studies by Kim *et al*., Gluszek *et al*. and Fletcher *et al*. in 2002.[15,20,21] We found that tumor was located in the stomach in 45.4% cases. Similarly, Reith *et al*., also that found stomach followed by small bowel were the main sites for GIST location.[22] In our study, most of the tumors (41.5%, *n* = 32) were

Table 1: Clinicopathological parameters of gastrointestinal stromal tumors cases

Variables	Number of cases (%)
Age	
<40	13
≥40	64
Gender	
Male	45
Female	32
Site	
Stomach	35
Small intestine	30
Esophagus	3
Colon	5
Pancreas	3
Appendix	1
Presentation	
GI bleeding	25
Pain	21
Dysphagia	10
Lump	6
Obstruction	5
Dyspepsia	4
Vommiting	4
Constipation	1
Jaundice	1
Size	
≤2	5
>2 and≤5	32
>5 and≤10	27
>10	13
Mitosis	
<5/50 hpf	45
>5/50 hpf	32
Risk stratification	
Very low risk	10 (12.98)
Low risk	10 (12.98)
Moderate risk	19 (24.67)
High risk	35 (45.45)
Histological types	
Spindle cell	65 (87)
Epitheloid	5 (3.9)
Mixed	7 (9.1)
Necrosis	
Present	13 (16.9)
Absent	64 (83.1)
Metastasis	
Seen	7 (9.1)
Not seen	70 (90.9)
Reccurence	
Seen	73 (94.8)
Not seen	4 (5.19)
CKIT	
Positive	72 (93.5)
Negative	5 (6.4)

Contd...

Table 1: Contd...

Variables	Number of cases (%)
Dog 1	
Positive	77 (100)
Negative	0

GI – Gastrointestinal

in size range of >2–≤5cm and only 5 (6.5%) tumors were ≤2 cm. The mean size was 7.1 cm with a standard deviation (SD) of 5.36. Similar were the observations in studies from Saudi Arabia by Al Hussaini and US by Espinosa *et al*.[16,23,24] In contrast, in another study done by Sui *et al*. in China, 26/63 (41.26%) tumors were >5–≤10 cm in size, in 18 (28.57%) tumors size was >2–≤5 cm, in five patients size of tumor was >10 cm and in only four cases size of tumor was ≤2cm.[25] This disparity could be because of the early presentation of patients in our study. 10/77 (12.98%) cases were very low risk, 19/77 (24.67%) cases were low risk, 10/77 (12.98%) cases were moderate risk, and 35/77 (45.45%) cases were high risk. Our results were similar to the study done by Fletcher *et al*. in Harvard Medical School Boston, in which majority of cases were high risk, followed by low risk and finally intermediate risk represented as 41.94%, 32.26%, and 25.80%, respectively.[15] The most common histologic type in our study was spindle cell type in 67/77 (87% cases). Similarly, in a study by Miettinen *et al*. and Foo *et al*. spindle cell was the most common histological type.[14,26] In our study, necrosis was seen in 13/77 (16.88%) tumors and majority 7/13 (53.8%) cases of these tumors belonged to Group 6b. This is not in concordance with observations by Rebey and Abdel-Samie, where necrosis was seen in 64.7% cases. This could possibly be explained by lower percentage (45.45%) of high-risk cases in our study compared to theirs (85.4%).[27] Although in another Indian study by Lakshmi *et al*. study necrosis was seen in 25% of cases.[17] In our study, 4 (5.4%) tumors showed nodal positivity and 1 patient out of these belonged to Group 6a and rest belonged to 6b. Both these groups correspond to high risk. In a study done by Rebey and Abdel-Samie, 3/51 (5.8%) cases showed nodal positivity.[27] In some other studies by Blay *et al*. and Loong, nodal positivity was rarely seen.[28,29] In our study, metastasis was seen in 7/77 (9.1%) cases, and 57.1% of these cases belonged to Group 6a. Similar results were observed by Lakshmi *et al*.[17] In our study, 4/77 (5.19%) cases had a recurrence. In 50% of the patients showing recurrence were Miettinen group 6b. Our results are in accordance with Lillemoe and Efron and Boni *et al*. In our study, the tumor size >10 cm developed recurrence in 50% cases. This was similar to the study by Boni *et al*. patients in which with tumor size >10 cm in diameter developed disease recurrence more frequently (75%) than those with smaller tumor size. Furthermore, low number of mitotic count was found to correlate with less risk of recurrence.[30,31]

Discovered on gastrointestinal stromal tumor1 and CKIT

We found that CKIT was positive in 72/77 (93.5%) patients. Among the cases positive for CKIT, 49 (68.05%) showed 3+ staining, i.e. more than 50% tumor cells showed positive staining and 23 (31.94%) cases showed 2+ staining, i.e. 25%–50% of tumor cells showed positive staining. In spindle cell variant of GIST, CKIT was positive in 95.2% patients. In epithelioid variant of GIST, CKIT was positive in 75% cases. Hence, spindle cell GISTs were more sensitive to CKIT compared to epithelioid GISTs.

DOG 1 was positive in all 77 (100%) cases. Among DOG1-positive cases, 62 (80.5%) cases showed 3+ staining intensity and 15 (19.48%) showed 2+ staining intensity. It showed similar results in all morphological types of GISTs. Cases which were found to be negative for CKIT showed positive staining for DOG1. Mixed tumors showed C-KIT and DOG1 positivity in all cases. Our results are comparable to many other studies carried out across the globe. In a study done by Rebey and Abdel-Samie, out of the 51 cases of GISTs, 35/51 (68.6%) cases were positive for c-KIT and 48 (94.1%) cases were positive for DOG1 antibodies.[27] Thirteen cases were DOG1-positive c-KIT negative. In another study by Geramizadeh *et al*. in Iran, IHC for c-KIT was positive in all of the 50 cases, DOG-1 was positive in 39 (78%) cases and 11 (22%) of the cases were negative for DOG-1.[32] In the study by Espinosa *et al*. in Harvard Medical School DOG1 antibody identified 63 GISTs more than c-KIT and in the study by Liegl *et al.* DOG1 was positive in 36% of c-KIT-negative tumors.[16,19] while as Fatima *et al*. in Atlanta studied DOG1 utility in diagnosing GISTs on FNA. DOG1 was found to have 100% sensitivity and specificity in GIST cases. These studies demonstrated that DOG1 is a more sensitive marker for GIST than c-KIT.[33]

In our study, the expression of DOG 1 was not significantly different in various ages, sexes, positions tumor sizes, nuclear atypia, and histomorphologic types. This was in agreement with the study done by Espinosa *et al*. in US in 2008 and Sui *et al*. in Harbin medical university.[16,25] In contrast, Yin Mujun *et al*. reported that the expression was related to tumor position nuclear atypia and Fletcher *et al*. grade.

Summary and Conclusion

Considering the importance and at the same time situations of the diagnostic dilemma of GISTs, we undertook this retro-prospective study at our center both to study clinicopathologic spectrum and the relevance of the conventional marker CKIT and the novel marker DOG1 in the GIST cases. Broadly, our clinicopathologic features are in agreement with most other studies, and simultaneously, it enriches the literature base regarding CKIT and DOG1. We found that DOG1 does have an edge in the diagnosis of GIST particularly in cases which are CKIT negative. Therefore, our study suggests that DOG1 should be added

to work up of suspected cases of GIST It not only helps in resolving the dilemma about the diagnosis but it has prognostic implications as well, which is already a strong subject of contemporary research.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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