International Journal of Advances in Medicine
Edwin DGS et al. Int J Adv Med. 2022 Dec;9(12):1198-1202
http://www.ijmedicine.com
pISSN 2349-3925 | eISSN 2349-3933
DOI: https://dx.doi.org/10.18203/2349-3933.ijam20223003

Case Series

Expression of DOG 1 and correlation with Ki-67 and tumor characteristics in GIST: case series analysis in a tertiary care center

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Received: 31 October 2022
Accepted: 14 November 2022

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ABSTRACT

Gastrointestinal stromal tumours (GISTs) are the most common non epithelial, mesenchymal tumours of the gastrointestinal tract and amount to 1 to 3% of all gastrointestinal tumours. Histologically, GISTs demonstrate considerable morphologic variation. The aim of the study was to evaluate the histo-morphological features of GIST and the expression of DOG1 and KI-67 in these tumours. Eleven cases of GISTs received during a five-year period at a tertiary care centre were analysed for their demographic parameters, morphology and risk stratification. Immunohistochemistry for DOG1 and Ki67 was performed for all the eleven cases. In this study there was a female preponderance with the mid -fifties being the median age of presentation. The stomach and small intestine were the common sites of involvement. The histologic type was predominantly spindle cell with a few cases of mixed tumours. DOG 1 was positive in all the tumours and Ki-67 index was markedly elevated in the epithelioid cell type and in the high-risk category of tumours. DOG 1 holds good as an important marker for clinically suspected GIST diagnosis and Ki-67 expression correlates with the risk stratification of the tumour and can be a good prognostic factor.

Keywords: GIST, DOG1, Ki-67

INTRODUCTION

Gastrointestinal stromal tumours (GIST) are the most common mesenchymal tumours of the gastrointestinal tract and account for 1%-3% of primary gastric neoplasms. They represent less than 1% of all gastrointestinal tumours and approximately 25% of them are malignant.[1] Current incidence of GISTs is 15-20 per million.[2]

These tumours originate in the interstitial cells of Cajal (ICCs) which are cells of the autonomic nervous system and designated as the pacemaker cells of the gastrointestinal tract (GIT). The function of these cells is to signal the smooth muscles in the digestive system to contract and propel solids and liquid through GIT.[2] Majority of GISTs (60%) occur in the stomach, while about 30% are seen in the small intestine. Duodenal and rectal GISTs account for about 5% each and oesophageal and colonic GISTs are rare.[4] These tumours can occur in at any age but are rare in people younger than 40. The most commonly affected age group is 50-60 years.[1]

Histologically, GISTs demonstrate considerable morphological overlap with other tumours making it a diagnostic challenge. They are typically composed of spindle cells; a minor proportion (10-15%) has epithelioid features and some show a mixed cell histomorphology.[3,4] The diagnosis of GIST is based on the anatomic location of the tumour, histopathology and immunohistochemistry.

Majority of GISTs have mutation in the c-KIT proto-oncogene that encodes the CD117 protein, a transmembrane tyrosine kinase receptor.[2] Platelet derived growth factor receptor (PDGFRA) mutations are found in about 5% to 10% of sporadic GISTs and C-KIT negative cases.[4] DOG1 is a transmembrane calcium-activated chloride channel protein that is encoded by a gene called TMEM16A (TMEM16, FLJ10261, ANO1, ORAOV2, and AOS2) and is located on chromosome 11q13.[5] The

function of DOG1 is to regulate the cholinergic activity of gastrointestinal smooth muscle. It also activates RAS/RAF/MEK/ERK and the insulin-like growth factor (IGF)- dependent signalling pathways.[6] Thus, DOG1 plays a role in GIST development and progression, regardless of KIT and platelet-derived growth factor receptor alpha (PDGFRA) activation. DOG1 has been demonstrated to be positive in 89% of GISTs, including those that do not have CD117 or PDGFRA mutations.[7]

Ki-67 is a nuclear protein present in proliferating cells during G1, S, G2 and M phases. High Ki67 immunolabelling is considered as high risk in the risk stratification of GISTS. It also indicates a high recurrence rate of the tumour.[4]

This study was aimed at evaluating histo-morphological features of GIST and the expression of DOG1 and KI-67 in these tumours.

Materials

This was a retrospective review done at Saveetha medical college, a tertiary care centre and included 11 cases (9 resected specimens and 2 biopsies) diagnosed as GIST during a 5-year study period (July 2015 to October 2020). Details were obtained from the case records at the medical records division and histo-pathology registers in department of pathology. Demographic and clinic-pathological features [age, gender, risk group, mitotic count in 50 high power fields (HPFs), tumour size, tumour location, growth pattern, cellularity, nuclear pleomorphism, ulceration, necrosis and cell type] were obtained, tabulated, and analysed. Hematoxylin and eosin-stained slides perused, and slides were prepared from suitable paraffin blocks for performing immunohistochemistry.

The cases studied were divided into "lower-risk group", "high-risk group" and "intermediate risk group" based on tumour size and mitotic count in 50 HPFs.[16] Tumour diameter was expressed as ≤ 5 cm, 5-10 cm, >10 cm. Mitotic count was assessed in 50 HPF and were classified as < 5, 5-10, >10. Immunohistochemical staining was done according to standard protocols using the primary antibodies, DOG1 (Mouse anti human DOG monoclonal antibody, Pathinsitu Biotechnologies, DOG1.1) and Ki67 (Monoclonal mouse anti human Ki67 antibody, DAKO M7240). DOG1 staining was evaluated using the Allred scoring system i.e., staining intensity and percentage of positive cells, with a score being given using both. Staining intensity was reported as, 0=negative; 1=weak/ trace;2= moderate; 3=strong. The percentage of positive cells were given as 0=normal cells; 1=≤1%; 2=1-10%; 3=11-33%; 4=34-66%; 5=67-100%. The final score was obtained on addition of both the above score and was reported as: score 0=negative, score1-3=weak, score4-6=moderate, score 7-8=strong. Ki-67 was evaluated in the hot spots by counting the total number of positively stained nuclei of the tumour cells and the total number of tumour cells in each field. The Ki-67 index was given as a percentage i.e., the number of positive tumour cells in each HPF/total number of tumour cells in that field x 100. Ki-67 positive patients were divided into groups as less than 5% positivity, 5-10% positivity and >10% positivity. Ki67 >10% positivity was indicated as belonging to the high-risk category.

DOG1 expression and Ki67 labelling were done and compared with the histopathological features. SPSS version 19 was used to analyse the data. The demographic variables were analysed using descriptive statistics.

CASE SERIES

Demographic and clinical characteristics

A total of 11 cases of GIST were studied, and they comprised 9 resected specimens and 2 biopsies (Table 1). Their age ranged from 17-75 years and mean age was 56±15.4 years (Table 2). The 4 (44%) cases were male and 7 (66%) were female (Figure 1). In our series, 4 (36%) cases occurred in the stomach, 4(36%) cases in the small intestine, 2 (18%) cases in the colon and 1 in the retroperitoneum(extra-intestinal). (Figure 2, Table 3) The size of the tumours ranged from 1.5 cm to 30 cm with a median tumour diameter of 9.06±4.24 cm. In 23% of the cases the tumour size was >5-10 cm, in 29% it was >10 cm and in 48% it was <5 cm (Figure 3).

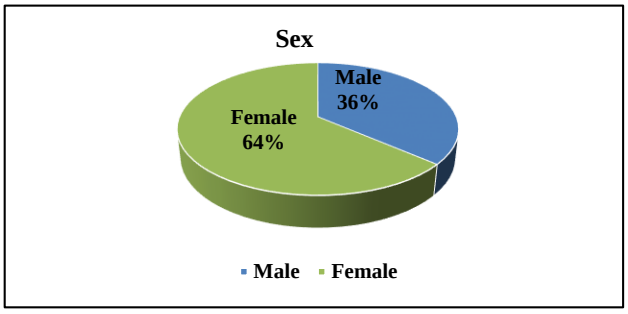

Figure 1: Gender wise distribution of GIST cases.

Figure 2: Gross picture of transverse colon GIST.

In the cases reviewed, most of them had presented with symptoms predominantly related to the gastrointestinal tract like vague abdominal pain, tightness, bloating, early satiety and altered bowel habits lasting over months. Along with other prodromal symptoms of cachexia like weight loss, loss of appetite and low-grade fever.

Table 1: Demographic and pathological characteristics of the 11 GIST cases.

Age (Years)/ sex	Site	Type	DOG1 expression (Intensity, proportion)	Ki-67 expression (%)
75/F	Rectum	Spindle cell type	Moderate, 75%	7
62/F	Ileum	Spindle cell type	Moderate, 90%	2
51/F	Gastric	Mixed	Moderate, 75%	Nil
65/M	Gastric	Mixed	Strong, 70%	10
67/F	Gastric	Spindle cell type	Mild, 70%	1
65/F	Broad ligament	Mixed	Moderate, 80%	3
60/F	Gastric	Spindle cell type	Strong, 80%	28
59/F	Jejuneum	Spindle cell type	Moderate, 80%	23
42/M	Ileum	Spindle cell type	Moderate, 90%	1.4
17/M	Transverse colon	Epithelioid cell type	Mild, 80%	53
59/M	Jejuneum	Spindle cell type	Strong, 95%	2

Table 2: Age wise distribution of cases of GIST.

Age group (years)	No. of cases
<20	1
21-30	0
31-40	0
41-50	1
51-60	4
61-70	4
>70	1
Total	11

Table 3: Site wise distribution of GIST cases.

Sites	No. of cases
Stomach	4
Ileum	2
Jejunum	2
Rectum	1
Transverse colon	1
Extra intestinal	1
Total	11

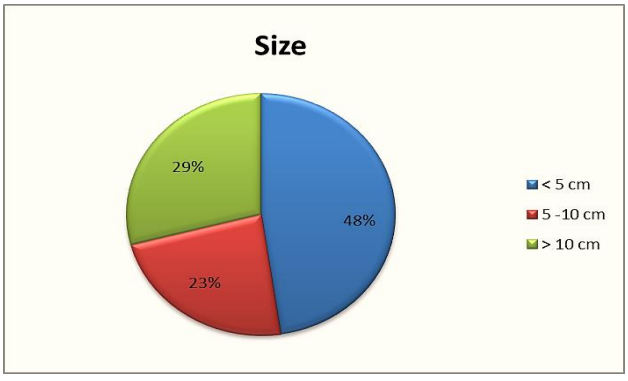

Figure 3: Distribution of GIST cases according to size.

Histomorphology and immunohistochemical expression

Histopathologically, 7 cases (63%) exhibited the spindle cell type morphology predominantly, 3 (27%) cases had mixed cell type morphology, 1 (9%) case had pure epithelioid cell morphology. Mitotic count varied from 0 to 50 with a (mean=7.4±15.3) per 50 high-power fields (HPFs). Using the criteria of Fletcher et al, namely tumour size and mitotic index, 7 (63%) cases were classified as high-risk, 3 cases (27%) were classified as intermediate-risk and 1(9%) case was classified as low-risk (Figure 4).

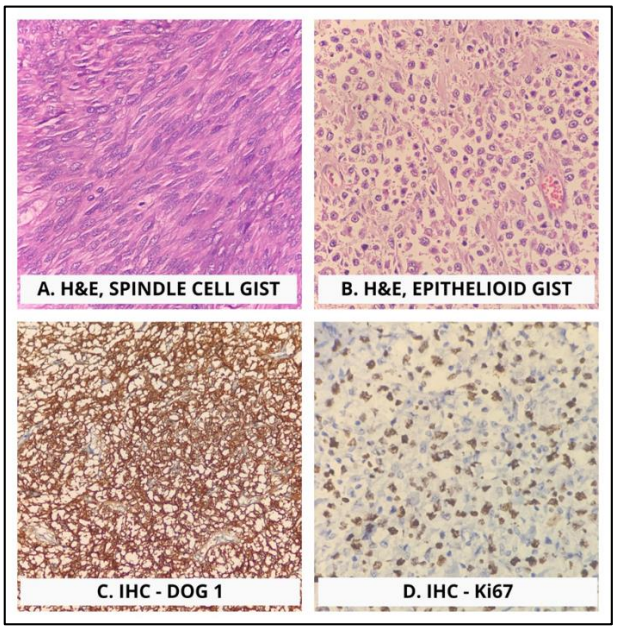

Figure 4 (A-D):40X, H and E stain showing spindle cell GIST. 40X, H and E stain showing epithelioid GIST. IHC DOG1, 40X, case of epithelioid GIST showing strong membrane and cytoplasmic positivity. IHC Ki67, 40X, case of epithelioid GIST showing nuclear positivity in 50-55% of tumor cells.

DOG-1 was positive in all the cases-100% positivity. 6 (54%) cases showed moderate intensity staining, 3 (27%) cases exhibited strong positivity and 2 (18%) cases showed mild positivity. The Ki-67 positivity index varied from 0.2% to 53%. Mean Ki-67 positivity in the spindle cell type was 9.2% and 4.5% in the mixed cell type. The lone epithelioid cell type had a very high Ki67 index of 53% (Table 4). Mean Ki-67 positivity in the high-risk group

was 17.8%; in the low risk and moderate risk groups the Ki-67 positivity was 2% each (Table 5).

Table 4: Correlation of Ki67 index with the cell type of GIST.

Cell type	Ki-67 <5%	Ki-67 6-10%	Ki-67 11-30%	Ki-67 >30%
Spindle cell type	4	1	2	0
Epithelioid type	0	0	0	1
Mixed cell type	2	1	0	0

Table 5: Correlation of Ki67 index with risk stratification.

Variables	Ki-67 <5%	Ki-67 6-10%	Ki-67 11-30%	Ki-67 >30%
High risk cases	2	2	2	1
Moderate risk cases	3	0	0	0
Low risk cases	1	0	0	0

DISCUSSION

GISTs are the most common mesenchymal tumours of the digestive system and occur most commonly in the 6th and 7th decades.[11,12] In the present study too, the median age was found to be in the fifties (Median 56). In a study by King et al a male to female ratio of 2:1 was demonstrated, but in our study there was a slight female preponderance.[13]

Although GISTs can occur anywhere along the digestive tract, they are most often seen in the stomach (50-60%) followed by the small bowel (25-30%), colon-rectum (5-15%) and oesophagus (2%).[13-16] In our study there were an equal number of cases in the small intestine (4 cases) and the stomach (4 cases). It has been reported that tumour localization is an independent prognostic marker apart from age, tumour size and mitotic rate.[15]

The size of the tumours varied from 1.5 cm to 30 cm with a median size of 9.06 cm in the present study. Similar findings have been reported in a study by Sahin et al.[3]

In a study of 127 GIST cases, by DeMaetto et al the cellular morphology was predominantly spindle-shaped in 112 cases (88%), similar to our study wherein the spindle cell morphology was the predominant microscopic type (7/11) (63%). The next most common type was the mixed cell type (3/11) (27%) and the epithelioid cell type was the least common (1/11) (9%).[21]

As per literature, the cases are sub classified into risk groups which aids in predicting the prognosis and malignant potential of GISTs.[9,10] In this study, we have used the risk assessment of Fletcher et al also mitotic count, tumour size, anatomic location, tumour necrosis, and nuclear pleomorphism have also been shown to be the prognostic parameters for GIST as per literature from other studies.[9,10] Similar to the literature, necrosis, high mitotic count, high cellularity, greater tumour size and high nuclear atypia were also detected to be associated with high-risk group in the present study.

Several studies have shown DOG1 immunostaining to have a high specificity and sensitivity in the diagnosis of GIST.[14-16] Similarly, in our study too, DOG-1 positivity was found to be 100%.

Beside immunohistochemical markers like DOG-1, Ki-67 immunolabelling is strongly recommended while diagnosing a GIST, as high Ki-67 proliferation index is widely considered as an indicator of poor outcome.[12,18] Ki-67 proliferation index over 10% has been reported to indicate a poorer outcome.[1,16,17] In the present study, Ki-67 proliferation index of more than 10% was found to be associated with the high-risk group. There was also a direct association of low Ki-67 positivity and the low-risk group. Increased expression of Ki-67 is helpful in predicting the malignant behaviour of tumours.[18,19]

CONCLUSION

GISTs are rare tumours which can have an aggressive behaviour. DOG1 positivity is a highly reliable biomarker for these tumours, and it is imperative that it is performed in all suspected cases of GIST. In addition, Ki67 index appears to be directly proportional to increasing risk stratification and thus will help in prognostication. Hence it is suggested that the above two biomarkers be performed routinely in all cases of GIST, thus improving the accuracy of diagnosis and management of these tumours

ACKNOWLEDGEMENTS

Author would like to thank the faculty and the supporting staff of the department of pathology, Saveetha medical college for all the help rendered by them.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Sbaraglia M, Businello G, Bellan E, Fassan M, Dei Tos AP. Mesenchymal tumours of the gastrointestinal tract. Pathologica. 2021;113(3):230.
2. Alshok MM. A clinical study on GIST in Babylon. 2020;1(53):001-5.
3. ŞAhin S, Ekinci Ö, SEçkin S, DUrSUn A. The diagnostic and prognostic utility of DOG1 expression on gastrointestinal stromal tumors. Turk Patoloji Derg. 2017;33(1):1-8.

4. Hirota S. Differential diagnosis of gastrointestinal stromal tumor by histopathology and immunohistochemistry. Translational Gastroenterol Hepatol. 2018;3.
5. Rizzo FM, Palmirotta R, Marzullo A, Resta N, Cives M, Tucci M et al. Parallelism of DOG1 expression with recurrence risk in gastrointestinal stromal tumors bearing KIT or PDGFRA mutations. BMC Cancer. 2016;16:87.
6. Duvvuri U, Shiwarski DJ, Xiao D, Bertrand C, Huang X, Edinger RS et al. TMEM16A induces APK and contributes directly to tumorigenesis and cancer progression. Cancer Res. 2012;72:3270-81.
7. Simon S, Grabellus F, Ferrera L, Galietta L, Schwindenhammer B, Mühlenberg T et al. DOG1 regulatesgrowth and IGFBP5 in gastrointestinal stromal tumors. Cancer Res. 2013;73:3661-70.
8. Mantese, George Gastrointestinal stromal tumor. Curr Opinion Gastroenterol. 2019;35(6):555-9.
9. Xiao CC, Zhang S, Wang MH, Huang LY, Wu P, Xu Y et al. Clinicopathological features and prognostic factors of rectal gastrointestinal stromal tumors. J Gastrointest Surg. 2013;17:793-8.
10. Burkill GJ, Badran M, Al-Muderis O, Meirion Thomas J, Judson IR, Fisher C et al. Malignant gastrointestinal stromal tumor: distribution, imaging features, and pattern of metastatic spread. Radiology. 2003;226:527-32.
11. Tryggvason G, Kristmundsson T, Orvar K, Jónasson JG, Magnússon MK, Gíslason HG. Clinical study on gastrointestinal stromal tumors (GIST) in iceland, 1990-2003. Dig Dis Sci. 2007;52:2249-53.
12. Nilsson B, Bümming P, Meis-Kindblom JM, Odén A, Dortok A, Gustavsson B et al. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era-a population-based study in western Sweden. Cancer. 2005;103:821-9.
13. King DM. The radiology of gastrointestinal stromal tumours (GIST). Cancer Imaging. 2005;5(1):150.
14. Lee CH, Liang CW, Espinosa I. The utility of discovered on gastrointestinal stromal tumor 1 (DOG1) antibody in surgical pathology-the GIST of it. Adv Anatomic Pathol. 2010;17(3):222-32.
15. Lopes LF, West RB, Bacchi LM, van de Rijn M, Bacchi CE. DOG1 for the diagnosis of gastrointestinal stromal tumor (GIST): comparison between 2 different antibodies. Appl Immunohistochem Molecular Morphol. 2010;18(4):333-7.
16. Fletcher CD, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. Int J Surgical Pathol. 2002;10(2):81-9.
17. Artigiani Neto R, Logullo AF, Stávale JN, Lourenço LG. Ki-67 expression score correlates to survival rate in gastrointestinal stromal tumors (GIST). Acta Cir Bras. 2012;27:315-21.
18. Panizo-Santos A, Sola I, Vega F, de Alava E, Lozano MD, Idoate MA et al. Predicting metastatic risk of gastrointestinal stromal tumors: Role of cell proliferation and cell cycle regulatory proteins. Int J Surg Pathol. 2000;8:133-44.
19. Noguchi T, Sato T, Takeno S, Uchida Y, Kashima K, Yokoyama S et al. Biological analysis of gastrointestinal stromal tumors. Oncol Rep. 2002;9:1277-82.
20. Belev B, Brèiæ I, Prejac J, Golubiæ ZA, Vrbanec D, Božikov J et al. Role of Ki-67 as a prognostic factor in gastrointestinal stromal tumors. World J Gastroenterol 2013;19:523-7.
21. Fletcher CD, Berman JJ, Corless C, Gorstein F. Lasota 9. J, Longley BJ et al. Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol. 2002;33(5):459-65.
22. DeMatteo. Two hundred gastrointestinal stromal tumours; recurrence patterns and prognostic factors for survival, Annals of Surgery. 2000;231(1):51-8
23. Arber A, Klein E, Bruce S, Sankoh S, Mohideen P. Metformin-glibenclamide versus metformin plus rosiglitazone in patients with type 2 diabetes inadequately controlled on metformin monotherapy. Diabetes Obes Metab. 2006;8(2):156-63.

Cite this article as: Edwin DGS, Diwagar NK, Sekhar G. Expression of DOG 1 and correlation with Ki-67 and tumor characteristics in GIST: case series analysis in a tertiary care center. Int J Adv Med 2022;9:1198-202.