Expression of DOG1, CD117 & PDGFRA in Gastrointestinal Stromal Tumours and Correlations with Clinicopathological Features & Risk Assessment

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ABSTRACT

BACKGROUND

Gastrointestinal stromal tumour (GIST) was first named in 1983. Gastrointestinal stromal tumours (GISTs) are a special kind of tumours which are derived from mesenchymal tissues of gastrointestinal tract and arises from the interstitial cells of Cajal, the pacemaker cells of the gastrointestinal (GI) tract responsible for the contractions of smooth muscles.¹ Determination of the type of mutations in GIST plays a major role in assessing the risk of progression of the disease and also allows determination of the clinical management and treatment. More accurate GIST diagnosis is possible by using simultaneously various types of antibodies to immunohistochemistry methods in routine procedures.²

METHODS

In this descriptive cross sectional study expression of DOG1, CD117 & PDGFRA was analysed in 70 patients with histopathologically diagnosed specimens of gastrointestinal stromal tumour, received in the Department of Pathology, Government Medical College, Thiruvananthapuram, using the immunohistochemical method.

RESULTS

On evaluating the CD117, DOG1 & PDGFRA expression in GIST by immunohistochemistry showed 71.4%, 84.3% and 55.7% positivity respectively. Most of the patients fall in the age group of 60 – 70 years with a slight male predominance. Most common location of GIST is stomach with tumour size of 5 – 10cm. On microscopic evaluation spindle type GIST was predominant histopathological type. Considering the risk groups, histological type, mitotic count and tumour size, PDGFRA expression is more in low-risk groups. PDGFRA expression has insignificant relation with clinicopathologic features including age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size. Relationship between positive expression by CD117 & DOG1 with risk group & site of lesion are not statistically significant. When compared to the similar studies in literature, the obtained results are concordant.

CONCLUSIONS

Our study concluded that, 71.4% positive immunoreactions for CD117, 84.3% positive immunoreactions for DOG1 & 55.7% positive immunoreactions for PDGFRA. PDGFRA expression has insignificant relation with clinicopathologic features including age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size. Relationship between positive expression by CD117 & DOG1 with risk group & site of lesion are not statistically significant. The importance of this study is that PDGFRA expression in tumours can be considered for treatment by using tyrosine kinase inhibitors and avapritinib. So PDGFRA testing in GIST show a new path in the targeted therapy.

KEY WORDS

GIST (Gastrointestinal Stromal Tumour), Discovered on GIST 1(DOG1), Cluster of Differentiation (CD117), Platelet Derived Growth Factor Receptor A(PDGFRA), Anoctamin 1(ANO1), Succinate Dehydrogenase (SDH), Risk Group, Mitotic Count.

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DOI: 10.14260/jemds/2022/20

How to Cite This Article:

Kunjunny RP, Ponnappan MC. Expression of DOG1, CD117 & PDGFRA in gastrointestinal stromal tumours and correlations with clinicopathological features & risk assessment. J Evolution Med Dent Sci 2022;11(01):103-108, DOI: 10.14260/jemds/2022/20

Submission 04-12-2021,
Peer Review 29-12-2021,
Acceptance 05-01-2022,
Published 25-01-2022.

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BACKGROUND

Gastrointestinal stromal tumour (GIST), a kind of rare tumour, is the most common mesenchymal tumour in the gastrointestinal tract.³ The concept of stromal tumour was first proposed by Mazur & Clark in 1983. Most GISTs have been previously classified as smooth muscles tumours (leiomyoma, leiomyosarcoma) or schwannoma due to incomplete understanding of its origin and differentiation.⁴⁻⁷ Presently, it is considered that GISTs originate from stem cells that differentiate toward interstitial cells of Cajal expressing tyrosine kinase receptors KIT or platelet derived growth factor receptor alpha (PDGFRA).⁸

The tumours can start anywhere in the GI tract, but they occur most often in the stomach (60%) or the small intestine (35%). Most of the rest are found in the oesophagus, colon & rectum. A small number develop in the abdomen outside the GI tract.⁹⁻¹¹

Localized GIST presents as a well circumscribed mass of highly variable size (ranging from incidental, submillimetre lesions to >20cm). In larger lesions, the cut surface may show foci of haemorrhage, cystic change, and/or necrosis. Gastric GISTs often feature an intraluminal component & may produce umbilicated mucosal ulcers.¹² In the small bowel, GISTs present as external masses. Some GISTs feature a narrow pedicle linked to the serosal surface, the interruption of which may contribute to the generation of extra gastrointestinal GISTs. Advanced disease most often presents as a main lesion associated with multiple smaller nodules that may extend from the diaphragm to the pelvis. Invasion of surrounding organs such as the spleen & pancreas can be observed in aggressive tumours. SDH deficient GISTs are often associated with a distinctive multinodular pattern of growth.¹³⁻¹⁶

GIST is slightly more common in men. Although GISTs are most often diagnosed in people at 50 years of age or older, they can occur in any age group.¹⁷

Smaller GISTs are detected incidentally during endoscopy, surgery or CT. Advanced GISTs spread into the peritoneal cavity & retroperitoneal space and often metastasize to the liver. Bone, skin & soft tissue metastases are frequently observed, whereas lung metastases are exceedingly rare.¹⁸⁻¹⁹ Systemic spread can occur years after detection of the primary tumour. Gastric GISTs exhibit a higher local recurrence rate than do small bowel GISTs, but the latter have a higher rate of abdominal dissemination & metastasis. Incidental subcentimetre GISTs (Called microGIST) seem to be remarkably common. Approximately 25% of gastric GISTs (excluding microGISTs) are clinically malignant.²⁰⁻²¹

The two most specific & sensitive diagnostic biomarkers for GIST are protein expression of KIT (CD117 – a transmembrane tyrosine kinase receptor) and anoctamin 1 (ANO 1) (DOG 1), which are positive in about 95% of all GIST.²²

DOG1 is a calcium dependent chloride channel protein. It has many significant functions such as regulation of the cholinergic activity of gastrointestinal smooth muscle & regulation of both the survival & proliferation of cells. Its expression indicates tumourigenesis.²³

A small subset of GIST stain negative for both KIT & ANO1. The combined detection of GISTs CD117 & PDGFRA can provide accurate diagnose evidences both in pathology & in clinic.²⁴ The probability that DOG1 may be a potential target for GISTs therapy makes it possible to develop new targeted

drugs and to identify the patients who can benefit from this therapy.²⁵

Most syndromic GISTs are SDH – deficient, including those associated with the non-hereditary Carney triad (GIST, pulmonary chondroma, paraganglioma) and the autosomal dominant Carney-Stratakis syndrome (GIST & paraganglioma in the context of SDH germline mutations). Rarely, GISTs are associated with neurofibromatosis type 1 (NF1); such cases are often multifocal & most are located in the small bowel. The extremely rare familial GISTs are caused by germline mutation of KIT or PDGFRA.²⁶⁻²⁹

Objectives

To find out the proportions of GIST specimen with expression of DOG1, CD117 & PDGFRA was our primary objective. Secondary objective was to find out correlations with clinicopathological features & risk assessment.

METHODS

Descriptive cross sectional study was conducted at the Department of Pathology, Government Medical College, Thiruvananthapuram. Study period was one year from date of approval. A 5-year analysis of histopathologically proved gastrointestinal stromal tumours received in a period of 2016-2019 was done.

Sample Technique and Size

Consecutive

Sample size is calculated using the formula-

$$n = (1.96)2q/d^2$$

p is prevalence of expression of PDGFRA in gastrointestinal stromal tumour, from previous studies.¹

q is 100-p p= 58.2 q= 41.8

d is 20% of p d= 20x58.2/100= 11.64

$$n = 1.96 \times 1.96 \times 58.2 \times 41.8 / 11.64 \times 11.64 = 68.97$$

70 specimens will be included in the study

Study Variables

1. Expression of DOG1, CD117 & PDGFRA.
2. Age, sex, site of lesion, tumour size, mitotic count, histology type, and risk group.

Data Collection Tool

1. Formalin fixed paraffin embedded tissue blocks
2. Haematoxylin and eosin-stained sections.
3. IHC marker- DOG1, CD117, PDGFRA
4. Hospital records.
5. A structural proforma will be used to collect data

Data Collection Technique

All the specimens are fixed in 10% neutral buffered formalin. After the process of cutting, biting, tissue processing, paraffin embedded blocks are made. Sections of 4-5 micrometre

thickness are cut from the blocks and stained with haematoxylin eosin staining. The slides are analysed under optical microscope and diagnosis is made as per WHO classification. Immunohistochemistry performed on all diagnosed specimens of GIST with DOG1, CD117 and PDGFRA. Risk score will be done based on tumour size and mitotic index.

Statistical Analysis

Data will be entered into excel sheet and analysis to be done using SPSS software version. Qualitative variables such as sex, site of lesion, risk group, histology type, CD117, DOG1 & PDGFRA expression will be expressed as proportion and quantitative variables such as age, tumour size, and mitotic index will be expressed as mean and standard deviation. Analysis of data will be done using chi-square test.

RESULTS

The expression of DOG1, CD117 & PDGFRA in GIST was studied and also evaluated whether there is any significant relation between PDGFRA positivity and clinicopathological characters like age, sex, site of lesion, risk groups, tumour size, histological type and mitotic count. Also find out any correlation with CD117 & DOG1 positivity with risk group and site of tumour.

On evaluating the CD117, DOG1 & PDGFRA expression in GIST by immunohistochemistry showed 71.4% (Refer Table 1 & Figure 1, 2, 3), 84.3% (Refer Table 2 & Figure 4, 5) and 55.7% (Refer Table 3 & Figure 6) positivity respectively.

CD117	Count	Percent
Negative	20	28.6
Positive	50	71.4

Table 1. CD117 Expression

DOG1	Count	Percent
Negative	11	15.7
Positive	59	84.3

Table 2. DOG1 Expression

Most of the patients fall in the age group of 60 – 70 years with a slight male predominance. Most common location of GIST is stomach (Refer Table 4 & Figure 7, 8) with tumour size of 5 – 10cm.

PDGFRA	Count	Percent
Negative	31	44.3
Positive	39	55.7

Table 3. PDGFRA Expression

Site of Tumour	Count	Percent
Stomach	47	67.1
Small intestine	18	25.7
Large intestine	1	1.4
Pancreas	1	1.4
Mesentery	3	4.3

Table 4. Tumour Location

Figure 7. Tumour Location

Figure 8. Gross Appearance of GIST

On microscopic evaluation spindle type GIST was predominant histopathological type (Refer Table 5 & Figure 9, 10).

Histological Type	Count	Percent
Spindle	61	87.1
Epithelioid	6	8.6
Mixed	3	4.3

Table 5. Histologic Type

Figure 9. Histologic Type

Figure 10. Spindle Cells with Paranuclear Vacuoles

Majority of GIST cases comes under intermediate risk groups (35.7%), followed by low risk (30%) and high risk (28.6%) groups respectively.

Considering the risk groups, histological type, mitotic count and tumour size, PDGFRA expression is more in low-risk groups (68%), epithelioid and mixed type (66.7%) of GIST, 5 – 10 mitotic counts per 50 HPF (66.7%) and >10 cm tumour size (63.2%) respectively. PDGFRA expression has insignificant relation with clinicopathologic features including age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size (Refer Table 7). Relationship between positive expression by CD117 & DOG1 with risk group & site of lesion are not statistically significant as the p values are 0.211 and 0.317 (>0.05) respectively. (Refer Table 6). When compared to the similar studies in literature, the obtained results are concordant.

		Either or both CD117 & DOG1 Negative		Both CD117 & DOG1 Positive		χ^2	P
		Count	Percent	Count	Percent		
Risk groups	Very low/ low risk	12	48.0	13	52.0	3.11	0.211
	Intermediate risk	12	48.0	13	52.0		
	High risk	5	25.0	15	75.0		
Site of lesion	Stomach	21	44.7	26	55.3	2.3	0.317
	Small intestine	5	27.8	13	72.2		
	Others	3	60.0	2	40		

Table 6. Relationship between Positive Expression by CD117 & DOG1 with Risk Group & Site of Lesion

	Variable	Number	Positive	Percentage	χ^2	P
Age	< = 40	10	6	60.0%	2.6	0.272
	41 – 60	29	19	65.5%		
	>60	31	14	45.2%		
Sex	Male	38	23	60.5%	0.78	0.377
	Female	32	16	50.0%		
Site of lesion	Stomach	47	27	57.4%	0.56	0.757
	Small intestine	18	10	55.6%		
	Others	5	2	40.0%		
Risk groups	Very low/ low risk	25	17	68.0%	2.6	0.273
	Intermediate risk	25	13	52.0%		
	High risk	20	9	45.0%		
Histologic type	Spindle	61	33	54.1%	0.5	0.778
	Epithelioid	6	4	66.7%		
	Mixed	3	2	66.7%		
Mitotic count	<5	47	26	55.3%	1.06	0.590
	5 – 10	12	8	66.7%		
	>10	11	5	45.5%		
Tumour size	<2	2	1	50.0%	0.59	0.744
	2 – 5	19	10	52.6%		
	5 – 10	30	16	53.3%		
	>10	19	12	63.2%		

Table 7. The Relationship between PDGFRA Expression and Clinicopathologic Features

DISCUSSION

GISTs are common kind of tumours derived from gastrointestinal mesenchyme, which is presented as spindle and epithelial cells, and sometimes pleomorphic cells arranged in cords or diffuse histologically. GISTs express the gene product KIT (CD117) of c-Kit, and are a mutant c-Kit or PDGFRA induced tumour. CD117 can be a target for therapy, and the use of TKIs for the inhibition of KIT and PDGFRA kinase has revolutionized the treatment of GISTs, so this specific diagnose is a potential therapy of patients. Various prognostic factors for determining the survival for GIST include location, tumour size, risk group, mitotic rate and mutational status.

The present study included 70 GIST specimens received in the Department of Pathology, Government Medical College, Thiruvananthapuram from 2016 to 2019.

Out of 70 cases taken, most of the cases are in the age group of 60 – 70 years and shows a slight male predominance.

The most common location of tumour in the present study is the stomach. All other studies showed a similar finding except one study by Beril guler et al. in that small intestine is the most common site.

In the present study most common histological type of GIST is spindle type. All other studies showed similar findings.

Tumour size and mitotic rate have been accepted as the most reliable parameters by many authors for determining prognosis. The number of mitosis indicates active growth in the tumour. Tumour size and mitotic rate are used for categorizing the risk groups. 5 or fewer mitosis/50 HPF is associated with low- risk groups. In the present study majority of GIST cases have 5 – 10cm in size, mitotic count <5/50hpf and belongs to intermediate risk category. All other studies showed a similar finding except one study by Beril guler et al. in that majority cases belongs to high-risk category (54.1%).

The study of expression of DOG1, CD117 & PDGFRA in GIST was the primary objective of the present study.

The CD117, DOG1 & PDGFRA positivity in various studies ranges between 70 to 95% (CD117), 85 to 90% (DOG1) and 53 to 80% (PDGFRA) respectively. All studies showed increased positivity of CD117 & DOG1. The present study also shows the similar finding.

The secondary objective of the present study was to evaluate correlation between PDGFRA positivity and clinicopathological characters like age, sex, site of lesion, risk groups, tumour size, histological type and mitotic count. Also find out any correlation with CD117 & DOG1 positivity with risk group and site of tumour. The evaluation is done by calculating p value through chi square test. Relationship between positive expression by CD117 & DOG1 with risk group & site of lesion are not statistically significant as the p values are 0.211 and 0.317 (>0.05) respectively.

PDGFRA expression has insignificant relation with clinicopathologic features including age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size. The p value for age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size in the present study is in concordance with previous studies.

The Observations Made in the Present Study Are

1. The common age prevalence of GISTs falls under 60 – 70 years.
2. There is a slight male predominance.
3. Most common location of tumour is noted in stomach.
4. The most common histological type is spindle type.
5. Most of GISTs are comes under intermediate risk group.
6. Majority of tumours shows 5 – 10cm in size and mitotic count <5/50HPF.
7. The important observation made is that of expression of CD117, DOG1 and PDGFA, showed 71.4%, 84.3% and 55.7% respectively.
8. Considering the risk groups, histological type, mitotic count and tumour size, PDGFRA expression is more in low-risk groups.
9. There is no significant relation with clinicopathologic features including age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size.

CONCLUSIONS

Our study concluded that, 71.4% positive immunoreactions for CD117, 84.3% positive immunoreactions for DOG1 & 55.7% positive immunoreactions for PDGFRA. PDGFRA expression has insignificant relation with clinicopathologic features including age, sex, site of lesion, risk groups, histologic type, mitotic count and tumour size. Relationship between positive expression by CD117 & DOG1 with risk group & site of lesion are not statistically significant. The importance of this study is that PDGFRA expression in tumours can be considered for treatment by using tyrosine kinase inhibitors and avapritinib. So PDGFRA testing in GIST shows a new path in the targeted therapy.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

Financial or other competing interests: None.

Disclosure forms provided by the authors are available with the full text of this article at jemds.com.

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