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Original Article

Pathology Section

Clinico-Histomorphological Spectrum and CD34 in Gastro-Intestinal Stromal Tumours: An Experience from Tertiary Care Centre, Kolkata, India

SARBASHIS HOTA[1], SUKANYA GHOSH[2], TUSHAR KANTI DAS[3], ANJALI BANDYOPADHYAY[4]

ABSTRACT

Introduction: Gastrointestinal Stromal Tumours (GISTs) are an important subcategory of mesenchymal tumours of gastrointestinal tract. The discovery of c-kit mutation in a subset of GIST has made amenable the treatment of this entity by targeted therapy. Although, Cluster of Differentiation 34 (CD34) is a well-established marker aiding in diagnosis of GIST, recent development of novel markers like Discovered on GIST1 (DOG1), CD117 have undermined its value. Still it's a frequently used marker in the resource poor settings.

Aim: To study the expression of CD34 immunomarker with respect to site, grade, stage, histomorphological type and risk category of GIST specimens received in the stipulated time period.

Materials and Methods: An observational retrospective cross-sectional study was conducted in the Department of Pathology at R G Kar Medical College and Hospital, Kolkata, West Bengal, India. The duration of the study was three years and 11 months, from September 1, 2019 to August 31, 2022. All the samples diagnosed as GISTs within the study period, were taken from the received specimens in the department and immunohistochemical examination was done on the selected samples using monoclonal antibodies against CD34 after obtaining thin sections from formalin fixed paraffin embedded blocks and retrieval of antigen. The data was interpreted by light microscopy using a semi-quantitative method with respect to prefixed parameters, where 50% proportional positivity of CD34 in the tumour cells was considered as positive. The data was analysed using Statistical Package for Social Sciences (SPSS) version 25.0.

Results: Mean age of the study participants was 49.78 years. A total of 8 (34.7%) cases originating from stomach and 13 (56.5%) from intestine. Eight out of 23 (34.7%) cases showed positive expression of the marker. Six out of eight cases of gastric GISTs were found to be positive and 66.6% cases of high grade GISTs were positive for CD34. Statistically significant association was found between expression of CD34 and the site of tumour-GISTs arising from stomach, particularly of spindle cell type, showing strong expression (p=0.003). It was found that, high grade GISTs are more likely to be positive for CD34. None of the epithelioid GISTs have shown positivity, neither any significant association was evident between expression of this marker with tumour stage or risk category.

Conclusion: The GISTs arising from stomach, particularly of spindle cell type, are more likely to show strong CD34 expression. Higher grade GISTs were found to be associated with positive CD34 expression in the present study, but no significant association was evident between expression of this marker.

Keywords: Cluster of differentiation 34, Immunohistochemistry, Risk stratification

INTRODUCTION

The GISTs are an important subcategory of mesenchymal tumours of gastrointestinal tract. Originating from the interstitial cells of Cajal, these tumours commonly present as an eccentric mass from the wall of gut, often without any mucosal involvement. The discovery of c-kit mutation in a subset of GIST has made amenable the treatment of this entity by targeted therapy [1]. Gain of function mutations of c-kit (exon 11 and exon 9) and Platelet-derived Growth Factor Receptor Alpha (PDGFRA) (exon 18, pD842V mutation) are found to be responsible for majority of GISTs- modulating the Rat Sarcoma Virus/Mitogen Activated Protein Kinase (RAS/MAPK) and Phosphoinositide 3 Kinase/Protein Kinase B/Mammalian Target Of Rapamycin (PI3K/AKT/mTOR) pathway [1]. Kit mutations are commonly associated with small intestinal tumours and have greater metastatic potential than those of PDGFRA mutation containing GISTs, which have a predilection towards involving stomach [2]. The genomic structure of tumours was found to have important prognostic significance, particularly kit exon 11 mutations, show very good response with imatinib.

The CD34 is a transmembrane phosphoglycoprotein, expressed in early haematopoietic stem cells and endothelial cells [3]. Along with GISTs, tumours like dermatofibrosarcoma protruberans and haemangiopericytoma are known to express CD34. Although, CD34 is a well-established marker aiding in diagnosis of GIST, recent development of novel markers like DOG1, CD117 have undermined its value [1]. Still it's a frequently used marker in the resource poor settings owing to its easy availability. Although, numerous studies have focussed on the expression of CD34 in GISTs [4-6], studies on this matter from perspective of Eastern India is lacking, as per the best of author's knowledge. And also, the differential expression of this marker in GIST, according to site and risk stratification has not been studied much. The present study aimed at evaluation of the efficiency of CD34 in diagnosis of GIST and study of its expression patterns with variables like site, grade, stage, histomorphological type and risk group and to study the clinico-histomorphological spectrum of GIST specimens received within the stipulated time period. Also, to categorise the cases as per the risk stratification endorsed by College of American Pathologists (CAP) [7];

- To study the clinico-histomorphological spectrum of GIST specimens received within the stipulated time period;
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MATERIALS AND METHODS

An observational retrospective cross-sectional study was conducted in the Department of Pathology at R G Kar Medical College and Hospital, Kolkata, West Bengal, India. The duration of the study was three years and 11 months, from September 1, 2019-August 31, 2022. The data analysis was done from 1st September, 2022 to 1st November, 2022. Study was approved by Institutional Ethics Committee (IEC) letter number (IEC Reg No ECR/322/Inst/WB/2013). The sample size thus, became 23. Thin sections of 2-3 micrometre were obtained from formalin-fixed paraffin embedded blocks.

Inclusion criteria: The resected tumour specimens of GI tract morphologically diagnosed as GISTs on histopathological examination, received within the study duration were included in the study.

Exclusion criteria: The core/incisional biopsy specimens of GIST and other forms of stromal tumours of alimentary tract were excluded from the study.

Study Procedure

Heat Induced Epitope Retrieval (HIER) procedure was done by microwave method using TRIS buffer, EMPARTA, pH 9.0. TRIS buffer (EMPARTA, pH 7.2) was used for washing. Endogenous peroxidise activity was blocked with PolyExcel peroxidase block, (PathnSitu) incubation with primary antibody (monoclonal antibody against CD34, EP88, PathnSitu) was done at 37°C for 60 minutes. For visualisation of result, serial incubation for 30 minutes each was carried out with PolyExcel target binder, PathnSitu; Poly HRP (PolyExcel Horseradish Peroxidase (HRP) Diaminobenzidine (DAB) detection system, PathnSitu) and chromogen (Polyexcel stunn DAB buffer and Polyexcel stunn DAB chromogen, PathnSitu). The staging, risk categorisation and grading was performed by protocol generated by CAP, which followed the recommendations of American Joint Committee on Cancer (AJCC), 8th edition for staging [4]. Grading was based on mitotic figure count per 50 high power fields.

STATISTICAL ANALYSIS

The data was interpreted by light microscopy using a semi-quantitative method (50% proportional positivity of CD34 in the tumour cells was considered as positive) [5] and statistical analysis was done by SPSS version 25.0. Chi-square test and Fisher's-exact test were used for calculating p-value and a p-value <0.05 was considered statistically significant.

RESULTS

Total 23 cases of GIST has been included in the study, among which 39.1 % were from males and rest were from females (60.9%). Mean age of cases were 49.78 years. Total 86.9% cases were spindle cell GISTs, whereas, only 3 (13.1%) cases were of epithelioid morphology (two from stomach and one extra-gastrointestinal). Two cases of gastric GIST were frankly metastatic, one involved liver and the other involved spleen. (both of the cases were histomorphologically spindle cell type and high grade) [Table/Fig-1].

Age (in years)	Site	Type	Grade	T stage	Risk category	CD34 status
50	Intestine	Spindle	Low	3	Moderate	Negative
60	Messentery	Spindle	Low	4	High	Negative
42	Intestine	Spindle	Low	4	High	Positive
42	Intestine	Spindle	Low	4	High	Negative
33	Intestine	Spindle	High	4	High	Negative
60	Intestine	Spindle	Low	3	Moderate	Negative
34	Intestine	Spindle	Low	2	Low	Negative
60	Intestine	Spindle	Low	3	Moderate	Negative
60	Retroperitoneum	Epithelioid	High	3	High	Negative
48	Stomach	Epithelioid	Low	2	Very Low	Negative
40	Intestine	Spindle	Low	3	Moderate	Positive
37	Stomach	Epithelioid	Low	2	Low	Negative
62	Stomach	Spindle	High	3	High	Positive
70	Stomach	Spindle	High	3	Moderate	Positive
55	Intestine	Spindle	Low	4	High	Negative
57	Stomach	Spindle	High	4	High	Positive
55	Stomach	Spindle	High	4	High	Positive
42	Intestine	Spindle	Low	3	Moderate	Negative
40	Intestine	Spindle	High	4	High	Negative
39	Stomach	Spindle	High	4	High	Positive
55	Intestine	Spindle	Low	3	Moderate	Negative
60	Stomach	Spindle	High	3	High	Positive
44	Intestine	Spindle	Low	4	High	Negative

[Table/Fig-1]: Representation of the age, site, grade, stage and risk category of tumours along with corresponding CD34 expression.

Three sites were detected for tumours, i.e., stomach, intestine and messentary/retroperitoneum. Out of which, stomach showed maximum CD34 positivity (six cases). Statistically significant association was found between CD34 expression with the site of GIST (p=0.003) [Table/Fig-2].

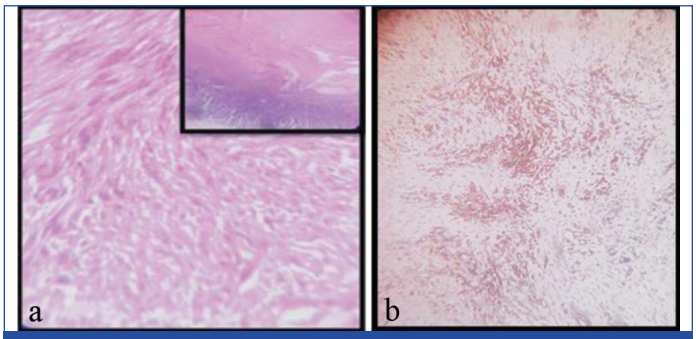
A total of 8 (40%) cases of spindle cell type GIST have shown positive expression of CD34 [Table/Fig-3-5].

Site of tumour	Positive	Negative	Total	p-value
Stomach	6	2	8	0.003
Intestine	2	11	13	
Messentery/retroperitoneum	0	2	2	
Total	8	15	23	

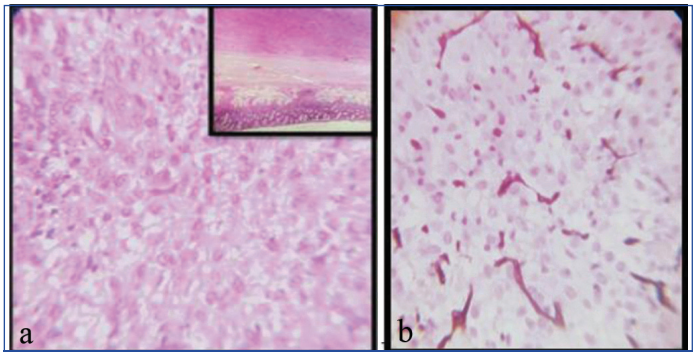
[Table/Fig-2]: Distribution of CD34 expression of cases with respect to site of tumour.

Histomorphological type	Positive	Negative	Total	p-value
Spindle cell	8	12	20	0.5257
Epithelioid	0	3	3	
Total	8	15	23	

[Table/Fig-3]: Distribution of CD34 expression of cases with respect to histomorphological type.



[Table/Fig-4]: a) Spindle cell GIST stomach, Haematoxylin and Eosin (H&E), 400X; Inset 40X; b) CD34 immunostain in the same, 100X.



[Table/Fig-5]: a) Epithelioid GIST, H&E, 400X; Inset 40X; b) Negative staining of CD34 in tumour cells, 400X.

Based on the mitotic figure evaluation, 60.8% cases were of low-grade while high grade tumour comprised 39.2% cases [Table/Fig-6]. AJCC staging is entirely based on the size of tumour. In the present study, 43.5% cases each presented with T3 and T4 stage, only 13% presented with T2 stage [Table/Fig-7]. Based on CAP endorsed risk stratification scheme, 4.3% were of very low risk, 8.6% low risk, 30.4% moderate risk, and 56.5% high risk cases were found [Table/Fig-8].

Grade	Positive	Negative	Total	p-value
Low	2	12	14	0.0228
High	6	3	9	
Total	8	15	23	

[Table/Fig-6]: Distribution of CD34 expression with respect to tumour grade.

Stage	Positive	Negative	Total	p-value
T2	0	3	3	0.685
T3	4	6	10	
T4	4	6	10	
Total	8	15	23	

[Table/Fig-7]: Distribution of CD34 expression with respect to tumour stage.

Risk category	Positive	Negative	Total	p-value
Very low risk	0	1	1	0.5257
Low risk	0	2	2	
Moderate risk	2	5	7	
High risk	6	7	13	
Total	8	15	23	

[Table/Fig-8]: Distribution of CD34 expression with respect to risk stratum.

DISCUSSION

Mean age of presentation of the 23 cases was found to be 49.78 years. A 60.9% cases were female with 39.1% cases from male population. The lowest age of presentation is at 33 years. In the fifth and sixth decade, the female cases have outnumbered male, whereas, it is equally distributed in the upper and lower extremes of ages. In contrary to the global prevalence pattern, in the centre, small intestinal GISTs (56.5%) have outnumbered the GISTs arising from stomach (34.6%). This finding is coherent with the report published by Sengupta R et al., from the same state in 2020, from other similar tertiary care Institutions [8]. This may be due to the fact that, intestinal GISTs often present early with features of intestinal obstruction, thus, are more often recognised than their gastric counterparts. This may have accounted for the over-representation of these cases in author's experience. The same study has expressed concern on the rising prevalence of GIST cases in India [6]. Additionally, two cases of extra-gastrointestinal GISTs (eGIST) were found; one arising from retroperitoneum and another located in mesentery.

The gross feature of presentation was also fascinating. Although, most cases presented as an eccentric mass arising from the wall of GI tract sparing the mucosa (often enormous in size); one case presented with a pedunculated globular mass hanging from outer aspect of the small gut. A particularly interesting case was that of a 37-year-old female patient, which presented as a dumbbell shaped mass in stomach, protruding into both mucosal and serosal aspect, piercing the muscularis propria. The case, removed by gastrostomy, was diagnosed histologically as epithelioid GIST. Overall, 34.7% cases have shown positive expression of CD34. In negative cases, the endothelial cells of the blood vessels served as positive internal control. Statistically significant association was found between expression of CD34 and the site of tumour; GISTs arising from stomach, particularly of spindle cell type, showing strong expression [Table/Fig-2-4]. This is concordant with the study of Hirota S, who found that, over 90% of gastric GIST of spindle cell type show positive expression of CD34 but, almost half of the spindle cell GISTs (other than gastric origin) show negative expression [Table/Fig-5] [4]. However, none of the epithelioid GISTs have expressed CD34 in the present study, whereas, about half of the epithelioid GISTs were positive in the study of Hirota S [4].

The findings are also congruent with that of Miettinen M et al., who analysed 96 cases of GIST (67 benign, six borderline and 23 malignant) and found that, the small intestinal tumours were more commonly CD34 negative [5,6]. The low total CD34 positive proportion (only 34.7%) apparent in the present study scenario is probably due to over-representation of the cases of small intestinal GISTs (70% of the benign spindle cell GISTs gave positive expression for CD34 in their study). Different aspects of diagnosis, molecular pathogenesis and therapeutic implications of GIST are elaborated by Koh Y et al., Robinson TL et al., Makar RR et al., Wu C-E et al., and Ceausu M et al., [9-13]. Kim KM et al., examined 747 cases of GISTs diagnosed between 2001 and 2002 [14]. The c-kit expression was found in 93.6% of cases and CD34 was positive in 80.1% cases. The positivity of CD34 was associated with higher risk of GISTs, according to their findings.

In the present study, however, higher grade GISTs have shown a propensity of positive CD34 expression (66.6% cases of high grade GIST were positive for CD34) [Table/Fig-6]. No significant association was evident between expression of this marker with tumour stage or risk category [Table/Fig-7,8]. Malik K et al., Rajappa S et al., Lakshmi VA et al., Iqbal N et al., Minhas S et al., have discussed various aspects of GIST from different centres of Indian subcontinent [15-19]. The disparities between findings from different studies with that of global literature, necessitate large scale studies from India for assessment of this disease, as aptly pointed out by Minhas S et al., [19].

Limitation(s)

The small number of specimens included in the study is the biggest limitation. The results need further confirmation from a wider study design. The relatively low prevalence of GIST as compared to the epithelial tumours of GI tract, requires a lengthier duration of study to get more cases. The relative expression of other newer markers, (along with that of CD34) could not be studied owing to financial constraint, which may have provided data of greater clinical importance.

CONCLUSION(S)

The GISTs arising from stomach, particularly of spindle cell type, are more likely to show strong CD34 expression, which is concordant with the findings of existing literature. Higher grade GISTs were found to be associated with positive CD34 expression in the present study; but, no significant association was evident between expression of this marker with tumour stage or risk category.

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PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Pathology, R G Kar Medical College and Hospital, Kolkata, West Bengal, India.
2. Senior Resident, Department of Pathology, R G Kar Medical College and Hospital, Kolkata, West Bengal, India.
3. Professor and Head, Department of Pathology, R G Kar Medical College and Hospital, Kolkata, West Bengal, India.
4. Professor, Department of Pathology, R G Kar Medical College and Hospital, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sarbashis Hota,
House No. 840/10, Raghunathpur, Jhargram, Kolkata-721507, West Bengal, India.
E-mail: sarbashishota94@gmail.com

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