Original Article

Definite Classification of Specific Gene Gastrointestinal Stromal Tumor (GIST) using the Immunohistochemical Technique 20 Cases in Rajavithi Hospital from January 2016 to December 2016

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Background: A Gastrointestinal Stromal Tumor (GIST) is a mesenchymal tumor of the gastrointestinal tract. It originates in the Interstitial Cells of Cajal (ICC), the pacemaker cells that produce mechanical muscle contractions. It can occur anywhere in the entire gastrointestinal (GI) tract, but it is most commonly found in the stomach, followed by the small bowel and colon, and there may also be extragastrointestinal involvement. Pathogenic mechanisms of GIST include Kit or PDGFRA proto-oncogene mutation which autostimulate Kit Tyrosine Kinase function. Mutational status can act as a prognostic factor for predicting specific response to treatment with tyrosine kinase inhibitors; consequently, GIST is classified into three groups: Kit Mutation; PDGFRA Mutation; and Wild-Type. The diagnosis of GIST relies heavily on the demonstration of specific tumor marker expressions (CD 117, CD34 and DOG1) using the Immunohistochemical Technique (IHC).

Objective: To classify GIST using the Immunohistochemical Technique (IHC)

Materials and Methods: Twenty paraffin-embedded tissue blocks of GIST were cut for immunostaining. The specific primary antibodies were CD117, CD34 and DOG1. The immunoreactivity was evaluated for classification of GIST.

Results: Eleven cases (55.0%) were diagnosed as Kit Mutation, two (10.0%) were PDGFRA Mutation, and seven cases (35.0%) were wild-type.

Conclusion: GIST is the most common mesenchymal tumor of the digestive tract. Definite classification of specific gene mutation with the IHC technique is a major step towards guidance for targeted therapy and prognosis.

Keywords: Gastrointestinal stromal tumor (GIST), Immunohistochemical technique (IHC), CD117, CD34, DOG1, Kit mutation, PDGFRA mutation, Wild-type mutation.

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A gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor in the gastrointestinal tract. It is quite rare, accounting for 0.1 to 3% of all gastrointestinal malignancies. The majority originate in the stomach (50 to 70%), followed by the small bowel (25 to 35%), and most of the other occurrences are found in the esophagus, colon and rectum, while a small number develop in the abdomen outside the gastrointestinal tract (GI tract)[1-4]. Around 60% of GIST incidences are found to be symptomatic, and indications include abdominal pain (74%), abdominal mass (72%), gastrointestinal bleeding (44%), and gastrointestinal obstruction (44%). GIST mainly affects middle-aged to elderly adults, typically in their 60s. The tumor is rare in people younger than 40, but it can occur in people of any age[5-8]. GIST remains silent until reaching a large size, and it is a disease that is frequently misdiagnosed, often initially classified as smooth muscle neoplasm including leiomyoma, leiomyoblastoma or sarcoma[9-11]. It typically originates in the interstitial cells of Cajal (ICC), which are located in the submucosal and myenteric plexus of the GI tract[12-14] and are the pacemaker cells that produce the slow mechanical muscle contractions responsible for moving food through the digestive tract. These cells are controlled by receptor tyrosine kinase genes: c-Kit gene or Platelet-derived growth factor receptor A (PDGFRA) gene which are located on the cell surface[15-18]. Mutation of the receptor gene is a pathogenic mechanism of GIST which is classified into three types (KIT, PDGFRA, and Wild-Type)[19-22], and responses to medications can vary by mutation type[17-19]. Most cases of GIST can be identified based on a combination of tumor location, histologic appearance, and the presence of KIT using the

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immunohistochemical technique (IHC)[8,22]. With regard to its histological features, GIST can be divided into three types: spindle cell type, epithelioid cell type, and mixed type[23,24]. The IHC markers for classification of GIST are KIT (CD117), CD34 and DOG1[25,26] which are used as panels for accurate diagnosis[27-30]. The targeted therapies used for GIST are tyrosine kinase inhibitors which are designed to block the action of a specific enzyme called tyrosine kinase, which plays a major role in the functioning of cells and is active in the cancer cells, promoting tumor growth and progression. The primary medications used are tyrosine kinase inhibitors such as imatinib which have been successfully used in the treatment of GIST[31,32]. The latest clinical practice guidelines in the US and Europe recommend adjuvant imatinib with standard dose (400 mg/day) for at least 1 year. Wild-type patients are not likely to benefit from standard-dose imatinib and require a high dose (800 mg/day). Sunitinib is the only second-line tyrosine kinase inhibitor (TKI) approved for use after imatinib failure due to its multi-kinase receptors' inhibitory function[33-35]. Routinely, almost all GIST cases in Rajavithi Hospital are distinguished from other gastro-intestinal mesenchymal tumors using a panel of antibodies such as SMA, S-100, and Desmin with just a few antibodies for GIST diagnosis such as CD117 or DOG1; however, a panel of antibodies is not used for classification of GIST. The present study aimed to classify GIST using a panel of CD117, CD34 and DOG1 with the IHC technique. The complete diagnosis of mutation status of GIST is a predictive factor of response to targeted therapy.

Materials and Methods

The protocol of this research was reviewed and approved by the ethics committee of Rajavithi Hospital (No. 088/2560). The present study was an applied research, cross-sectional study.

Specimens

Tissue-embedded paraffin blocks from the medical records of twenty patients diagnosed with GIST between January and December, 2016 were selected from the Department of Pathology at Rajavithi Hospital. Age ranged from 25 to 84 years (mean 52.2 years), and eight cases (40.0%) were male while twelve (60.0%) were female.

Immunohistochemistry

All of the specimens were cut at 3 to 5 µm, deparaffinized in xylene and rehydrated in a graded series of alcohol and fully-automated processes using IHC staining technique by Bench Mark XT Automated Slide Stainer. The primary antibodies used were PATHWAY Anti-c-kit (9.7); Ventana, (Cat. No. 790-2951), CONFIRM anti-CD34 (QBEnd/10); Ventana, (Cat. No. 790-2927); DOG1 (SP31) Rabbit Monoclonal Antibody; and Ventana, (Cat. No. 760-4590). After end of run, slides were dehydrated in graded alcohol, cleaned with xylene, and mounted with a permanent mounting medium. The slides were viewed on an Olympus BX43 light microscope (Olympus, Tokyo, Japan) and assessed for presence of IHC staining in tumor cells. The staining pattern of each antibody is shown in Table 1 and Figures 1 to 9. The interpretation of IHC profile staining for classification of type of GIST is shown in Table 2.

Statistical analysis

The sample size for the present study was twenty cases. The data were presented as percentages of each type. The IBM SPSS Statistics version 22.0 was used.

Results

The twenty GIST-diagnosed patients (eight men and twelve women; age range 25 to 84 years; median age at study entry 52.2 years) showed all types of GIST after

Table 1. Antibody staining pattern in GIST

Antibody	Staining pattern (brown-yellow in color)
CD117	Cytoplasm, Membrane
CD34	Membrane
DOG1	Cytoplasm, Membrane

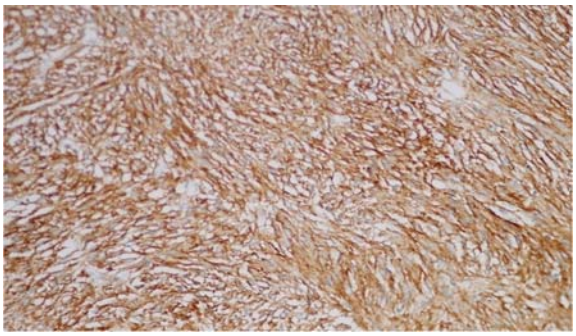

Figure. 1 GIST with Spindle cell type, CD117 positive stain, ob. 40x.

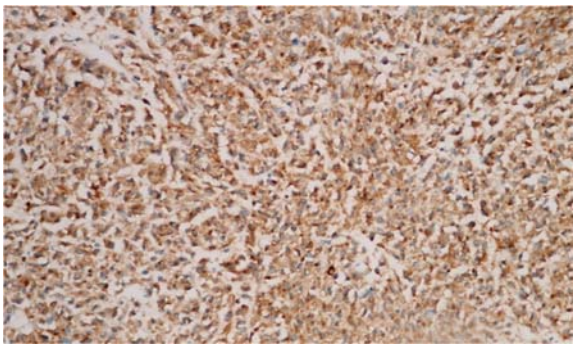

Figure. 2 GIST with Epithelioid type, CD117 positive stain, ob. 40x.

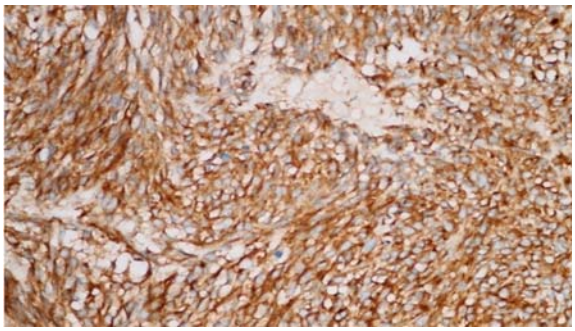

Figure. 3 GIST with Mixed cell type, CD117 positive stain, ob. 40x.

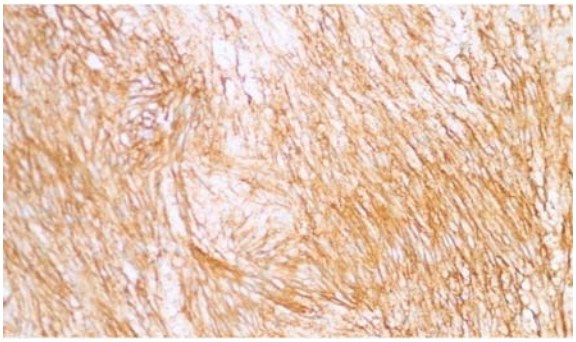

Figure. 4 GIST with Spindle cell type, CD34 positive stain, ob. 40x.

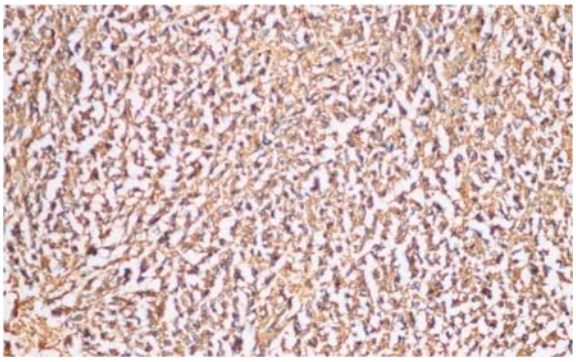

Figure. 5 GIST with Epithelioid type, CD34 positive stain, ob. 40x.

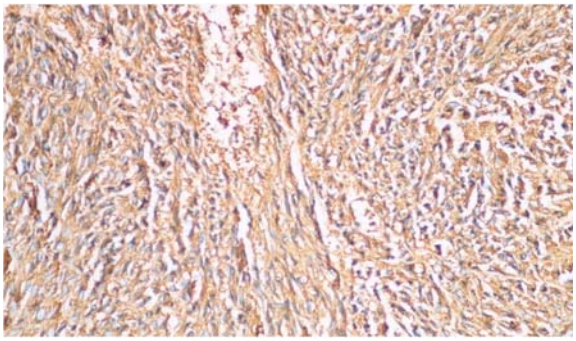

Figure. 6 GIST with Mixed cell type, CD34 positive stain, ob. 40x.

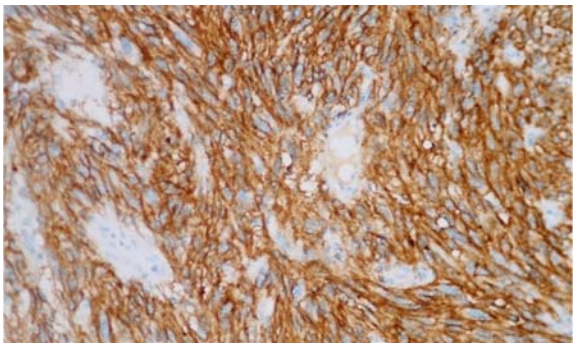

Figure. 7 GIST with Spindle cell type, DOG-1 positive stain, ob. 40x.

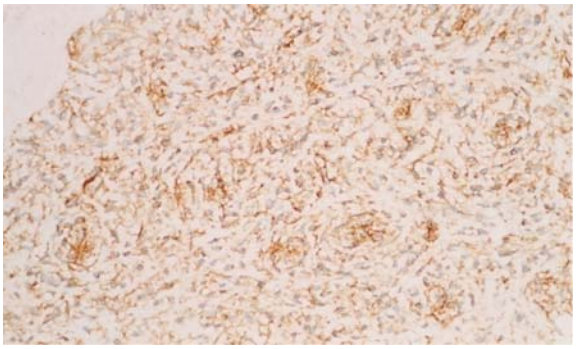

Figure. 8 GIST with Epithelioid type, DOG-1 positive stain, ob. 40x.

panel immunohistochemical staining was performed to classify type of GIST. The expressions of the antibody panel and the classification of GIST are shown in Table 3. Eleven (55.0%) cases were classified as Kit Mutation (CD117+, CD34+, DOG1+), two (10.0%) were classified as PDGFRA Mutation (CD117-, CD34-, DOG1+), and seven (35.0%) were wild-type (CD117+, CD34-, DOG1+). These are shown in Table 4 and Figure 10.

Discussion

GIST is a rare type of soft tissue sarcoma in the gastrointestinal system, accounting for 0.1 to 3% of all gastrointestinal malignancies. It is most commonly found in the stomach (60%) and the small intestine (30%), but it can occur outside the gastrointestinal tract in the mesentery, omentum and retroperitoneum, and these cases are known as extra-gastrointestinal GIST[1-4,36,37]. Microscopically

Table 2. Interpretation of immunohistochemical staining profile in GIST

Type of GIST	Antibody expression		
	CD117	CD34	DOG1
GIST, Kit Mutation	+	+	+
GIST, PDGFRA Mutation	-	-	+
GIST, Wild-Type	+	-	+

A known positive control of GIST was used with each slide

Table 3. Antibody panel expression and classification of GIST

Specimen No.	Antibody panel expression			Classification of GIST
	CD117	CD34	DOG1	
1	+	-	+	Wild-type
2	+	-	+	Wild-type
3	+	-	+	Wild-type
4	-	-	+	PDGFRA mutation
5	+	+	+	Kit mutation
6	+	+	+	Kit mutation
7	+	+/-	+	Wild-type
8	+	+/-	+	Wild-type
9	+	+	+	Kit mutation
10	+	+	+	Kit mutation
11	+	-	+	Wild-type
12	+	+	+	Kit mutation
13	+	+	+	Kit mutation
14	+	-	+	Wild-type
15	+	+	+	Kit mutation
16	+	+	+	Kit mutation
17	+	+	+	Kit mutation
18	-	-	+	PDGFRA mutation
19	+	+	+	Kit mutation
20	+	+	+	Kit mutation

Table 4. Percentages of GIST types (n = 20)

Types of GIST	Number	Percentage
Kit mutation	11	55.0
PDGFRA mutation	2	10.0
Wild-type	7	35.0

(histologically), the morphology of stromal tumors can sometimes look like a leiomyoma or a Schwannoma[36]. Most gastrointestinal mesenchymal tumors, which were previously, erroneously thought to be leiomyomas, schwannoma or leiomyosarcomas, are nowadays classified as GIST on the basis of molecular and immunohistochemical features[37]. The most important features in immunohistochemical differentiation of GIST from other similar soft-tissue tumors

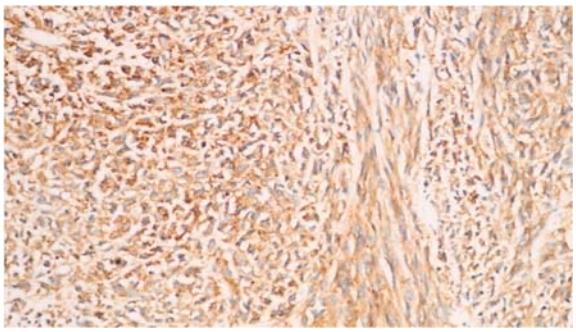

Figure. 9 GIST with Mixed cell type, DOG-1 positive stain, ob. 40x.

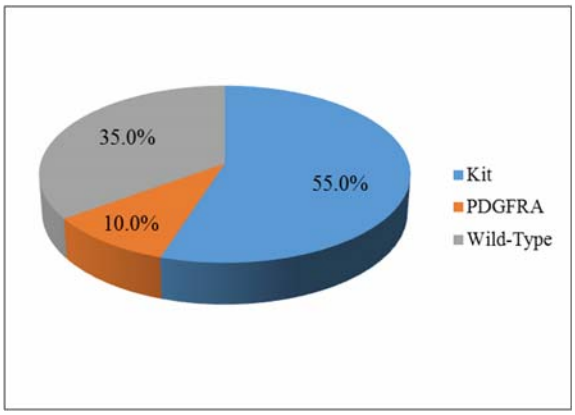

Figure 10. Molecular subsets of GIST.

are the antigens on the surface of the tumor cells[2]. IHC studies play a major rule in differential diagnosis and evaluation of appropriate immunophenotypic markers in context with morphology, and in most cases allow an accurate classification[38]. With the development of effective targeted therapies for GIST, the correct diagnosis and classification have a considerable clinical impact. KIT immunoreactivity has proved to be very useful in the treatment of GIST with immunotherapy as well as in diagnosis[39]. To make a diagnosis of GIST, IHC staining of the CD117 and CD34 is required because they can characteristically express both CD117 and CD34[40]. DOG1 is considered a very sensitive and specific marker for GIST that works in paraffin-embedded tissue and is highly expressed in Kit mutant and PDGFRA mutant GIST[27-29]; therefore, detection of CD117 and CD34 in combination with DOG1 increases the accuracy of GIST diagnosis and eventual guidance of individual patient therapy[28,29]. In the present study, the 20 GIST-diagnosed patients had a mean age of 52.15 years old with male predominance. To make a classification of GIST, IHC staining of the CD117, CD34 and DOG1 was required. They showed diffuse positive expressions, with eleven cases (55%) of

Kit Mutation, and two cases (10%) and seven cases (35%) of PDGFRA mutation and Wild-type respectively. These classifications were similar to those of previous studies which found that the large majority of GIST consisted of oncogenic Kit mutation (~80 to 85%), followed by Wild-type (~10 to 15%) and PDGFRA mutation (~5%)[2,16,18,41,42], but the percentages were different because of the limitation of time (one year) and sample size (just 20 participants because this is a very rare type of cancer) in the present study. A larger sample size over a period of many years could provide more accurate information.

Conclusion

GIST is the most frequent mesenchymal tumor of the gastrointestinal tract. Its most common location is the stomach, followed by the small bowel, the colorectum and the esophagus; however, it can also develop in the retroperitoneum, the omentum and the mesentery. Early diagnosis and treatment could save the lives of many patients who present with GIST. The type of mutation is prognostically significant for patients, and IHC staining is a useful aid in diagnosis. Mutational analysis is helpful in planning therapy because different mutations of GIST may affect prognosis and response to therapy.

What is already known on this topic?

Mostly general differential diagnostic features of GIST are distinguished from other soft tissue tumors by clinical, histopathological and some reactive antibodies of immunohistochemical features.

What this study adds?

This study demonstrated further development in the mutational status of GIST because histopathology and IHC techniques are more reliable in its diagnosis. Early diagnosis and classification of the mutational status of GIST with complete IHC staining and proper treatment could save the lives of many patients who present with GIST; however, molecular testing should be performed for treatment selection and assessment of disease progression. The molecular targets for therapeutic interventions are not only of importance in the treatment of GIST patients, but also in the development of novel drugs and new strategies in basic cancer therapy.

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Potential conflicts of interest

The authors declare no conflict of interest.

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