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Original Article

Histological and Immunological Evaluation of Gastrointestinal Stromal Tumors

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Abstract

Background: Gastrointestinal stromal tumors (GISTs) constitute the majority of gastrointestinal mesenchymal tumors. They usually express a proto-oncogen protein called CD117 detected by immunohistochemistry. This study investigated the differentiation of GISTs as well as the risk of aggressive behaviors in GISTs from surgically-treated patients in university affiliated hospitals.

Methods: The clinicopathologic, histomorphologic and immunohistochemical features of 36 GISTs of patients referring to two large general hospitals in the last 13 years were examined.

Results: The GISTs occurred in 36 patients (41.6% male and 58.4% female) aging 15 to 89 years. 50% of the cases were in the stomach, 30% in the small intestine and the remainder in the colon and mesenter. The diameters of tumors were 0.7 to 30 cm and mostly (60.6%) more than 5. 85% of tumor cells were spindle, 14% epithelioid, and the remainders were mixed. 36.1% of tumors showed mitotic counts > 5/50 HPF and 11.1% less than 5/50HPF. 33.3% of the tumors showed necrosis. 8 tumors had malignant behavior during 13-yrs follow up. Immunoreactivity for c-kit, SMA, chromogranin, synaptophysin, desmin and S100 were 83.3%, 69.4%, 44.4%, 41.6%, 50% and 0%, respectively. The decreasing rank order of differentiation forms were neural, smooth muscle, dual and null. Most of our tumors were in high risk group and most of the high risks were intestinal with neurogenic differentiation.

Conclusion: Immunostaining including c-kit is necessary to study the differentiation of gastrointestinal stromal tumors.
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Keywords ● Gastrointestinal stromal ● tumor ● histology ● immunohistochemistry ● c-kit

Introduction

Primary mesenchymal tumors arising in the wall of gastrointestinal (GI) tract are rare and heterogeneous, and their differentiation and biologic behavior are subjects of controversies.[1] Traditionally, the primary mesenchymal spindle cell tumors of the GI tract were almost uniformly classified as smooth muscle tumor such as lieomyoma, cellular lieomyoma or lieomyoblastoma and neurogenic like schwannoma.[2] The current classifications in the world health organization tumor series and the latest American forced institute of pathology books of tumors of GI tract refer to these tumors collectively as stromal tumors.[2]

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The term (GI stromal tumors (GISTs) was introduced as the main group of mesenchymal tumors of GI tract. According to recent evidences most GISTs are immunoreactive for CD117 (c-kit).[3]

The CD117, a proto-oncogen protein, has emerged as the most important defining feature and probably the gold standard for the diagnosis of GISTs.[3] In the GI tract, CD117-positive normal cells are the interstitial cells of cajal and autonomic nerve-related GI pace maker cells that regulate intestinal motility. Because of the immunohistochemical and ultrastructural similarities between cajal cells and GISTs, the histogenetic origin of GISTs was reported to be Cajal cells.[3] The potential availability of kit-tyrosine kinase inhibitor imatinib (Gleevec), as a target specific treatment, makes it important to properly recognized GISTs.[4] In this study the clinical, histomorphologic and immunohistochemical aspects of GISTs from patients referring to two university-affiliated hospitals were studied.

Material and Methods

This study is a retrospective analysis of 36 tumors of GI tract from patients referring to two Nemazee and Faghihi Hospitals affiliated with Shiraz University of Medical Sciences, Shiraz, Iran, in a period of 13 yrs starting from 1990. The tumor had originally been reported as GIST, lieomyoma, lieomyosarcoma, lieomyoblastoma, smooth muscle tumor of undetermined malignancy and neurogenic tumor of GI tract. The tumors were from various parts of the GI tract including stomach as well as small and large intestines.

Clinical data were reviewed from the hospital records. Tumor size and other gross findings were investigated from their pathology reports. A total of one to 25 slides for each case were evaluated. All slides were reviewed blindly by a pathologist without having any knowledge of immunohistochemistry (IHC) findings. Mitosis was counted in 50 consecutive fields of high power (X400) in the most cellular and mitotically active sites of the tumor. Moreover, cell type (spindle vs. epithelioid), necrosis, anaplasia, differentiation, vascular invasion, mucosal invasion, inflammatory cell infiltration and resected margins features were recorded for all cases.

Immunohistochemistry (IHC) experiments were performed on fresh sections of paraffin blocks of selected representative slides using avidin-biotin peroxidase complex detection system (from DAKO company, Denmark), with diaminobenzidine (DAB) as the chromogen. The primary antibodies for markers such as c-kit, SMA, S100, NSE, chromogranin, synaptophysin and desmin were used for these experiments. Staining was graded as 1+ to 3+ for the c-kit protein. After IHC studies medical charts of all the cases were compared and recorded.

Results

The mean age of the patients was 54.5±17.6 yrs (range 15-89 yrs). Out of the 36 tumor reviewed, 21 (58.4%) tumors were excised from female and the rest (41.6%) from male patients (Table1). The most common symptoms were abdominal pain in 16 patients (44.4%) followed by GI bleeding in 10 patients (28%). The rare symptoms were mass detection in six patients (16.6%) and intestinal obstruction in two patients (5.5%). Two cases were incidentally found i.e. the patient has been operated for an irrelevant cause and incidentally the surgeon has noticed a mass.

Table 1: Frequency of gastrointestinal stromal tumors (#) based on age, sex, site, histological subtypes (HS), size and immunohistochemistry differentiation (IHC) findings

Variables	Classes	#	%
Age (yr)	<50	12	*33.3*
	>50	24	*66.7*
Sex	Female	21	*58.3*
	Male	15	*41.7*
Site	Stomach	18	*50*
	Non stomach	18	*50*
HS	Spindle	29	*80.5*
	Epithelioid	5	*14.0*
	Mixed	2	*5.5*
Mitosis/50 (HPF)	= 0/50	19	*52.8*
	< 5/50	4	*11.1*
	> 5/50	13	*36.1*
Size (cm)	<5	13	*39.4*
	>5	20	*60.6*
IHC	Neural	12	*33.3*
	Muscle	8	*22.2*
	Non	6	*16.7*
	Dual	10	27.8

> Smaller; < larger; HPF= high power field

The locations of tumors were in the stomach (n=18), small intestine (n=11), large intestine (n=5) and the mesenteric (n=2). In eight patients multiple tumors were detected, four of which were in the small intestine i.e. there were several nodules of the tumoral tissue in the same organ. Most of the tumors were circumscribed. Their greatest diameter of the tumors ranged from 0.7 to 30 cm. However, in 3 cases the true size of tumors could not be determined as they had been sampled by biopsy. Twelve tumors, of which 8 were mitotically active, showed tumor necrosis. The sizes of cases with necrosis were 2.5 to 30 cm. Four tumors which were from intestine were larger than five cm, showed mucosal invasion. One small intestinal tumor was large (20 cm) and mitotically active, and was associated with vascular invasion.

The immunohistochemical finding has been summarized in Table 2. CD117 was documented in 30 cases (83.3%). The rate of c-kit positivity was highest among gastric GISTs (53.3%), followed by small intestine (26%), colon (10%) and abdomen (6.6%). The c-kit, however, was negative in six tumors with the histology of GISTs.The most reliable evidence of malignancy in our cases was omental or peritoneal metastasis. Omental spread was seen in six tumors. Mesenteric and liver metastasis was also present in two other tumors. Most of these tumors had originated from small intestine. All of these were more than five cm (6-20cm), and 50% were spindle with a mitotic rate of >5/50 high power field (HPF). Immunohistochemistry of these eight malignant GISTs showed mostly null differentiation. The least common form of differentiation was smooth muscle. The size of three tumors, out of eight tumors that did not show mitosis, was more than 10 cm.

Table 2: Frequency (#) and the type of immunoreactive antibodies of 36 gastrointestinal stromal tumors studied

Antigen positive	#	%
CD117	30	83.3
NSE	25	69.4
Chromogranin	16	44.4
Synaptophysin	15	41.6
SMA	18	50
Desmin	7	19.4
S100	0	-
Total	36	100

NSE= neuron specific enolase, SMA= smooth muscle antibody.

Table 3 shows the characteristics of tumors analyzed according to the risks of malignancy of GISTs defined by the National Institute of Health workshop.[5] Most of the tumors were in the high risk group. The predominant histology in the high risk group was spindle shape, which were mostly located in small intestine. The least differentiation in the high risk group was in the tumors from smooth muscle. More than half of the tumors in high risk group showed necrosis and anaplasia.

Discussion

The age of the patients was compatible with other previous studies.[1,4] The rate of tumors in female patients was higher in our patients in comparison with those reported in earlier studies.[1,4] The predominant location of GISTs was stomach as stated by previous studies.[3,5,7] Moreover, Symptoms varied according to the location and size of the tumors. In our patients the most common presenting symptom was abdominal pain as reported by Hashigva et al.[1]

The size of 20 out of 36 tumors studied was larger than five cm, median size of 10 cm, the same as (4.5 to 9.5 cm) of other reports.[1,8] We noticed necrosis in 33% of our GISTs due to their large size. It also worth mentioning that in our study the percentage of tumors with mucosal invasion was higher than (15% vs. 10%) those reported by Miettinen et al.[4]

Most of the GISTs seen in our study, like others,[1-3] were spindle shaped. Moreover, all of the epithelioid tumors were located in the stomach, as had been reported by Christopher and colleagues.[6] The rate of positive smooth muscle antibody (SMA) was 50% in our cases, whereas it was between 20 and 40% in cases reported by othes.[1-3]

The second most common type of differentiation seen in 10 out of 36 GISTs was dual, i.e. simultaneously positive (SMA), neuron specific enolase (NSE), chromogranin and synaptophysin. Whereas, in most of previous studies which were evaluating neural differentiation just one marker including chromogranin, synaptophysin or neurofilament was examined.[7] Although, the NSE reactivity was seen in 69% of our cases, it was cited as a nonspecific only in one case by Miettinen and colleagues,[2,3] Therefore, we do not recommend it as being a good marker for the evaluation of the neurogenic differentiation in GISTs.

The synaptophysin reactivity, which was not evaluated in previous studies, was 41% in the present study. None of our cases showed positive S100, as was noticed in the study of Miettinen and colleagues,[2,4] and somehow lower than the others which had 5% S100 positive GISTs.[3,7] Desmin positive reactivity was seen in 16% of our GISTs whereas, it was 2-16% in the reports of Miettinen and colleagues.[1,3] In the present study only two cases showed prominent nuclear anaplasia. Hence, we think nuclear anaplasia is not a common feature of GISTs which is in agreement with the earlier reports too.[2,3]

Table 3: Frequency of tumor characteristics according to the proposed risk of malignant vs. benign behavior.

Risk intensity	No	Age		Gender			Site		Cell Type			Diff		Necrosis	Anaplasia	
		<50	>50	M	F	S	NS	S	E	MI	NE	S	D			
Very low	2	-	2	2	-	1	1	2	-	-	-	2	-	-	-	
Low	6	2	4	2	4	3	3	5	1	-	4	1	1	2	1	
Intermediate	4	-	4	4	-	3	1	2	2	-	1	-	2	1	1	
High	21	10	11	5	16	8	13	17	2	2	6	3	6	8	15	

M= Male; F=Female in the sex column; S= Stomach and NS= Non stomach in the site column;
S= spindle; E= Epithelioid; MI= mixed in the cell type column. NE= Neurogenic,
S= Smooth muscle: and D= Dual in the differentiation (diff) column

In all of tumors, the surgical resected margins were free which showed that most of the GISTs were well defined and could be enucleated. Moreover, one of the tumors, which were more than 20 cm, showed vascular invasion with high mitotic counts more than 10/50 HPF mitosis and necrosis. More than 80% of the cases were reactive for c-kit. Our c-kit negative GISTs were equally located in stomach and intestine, all were spindle, and were of smooth muscle, neural, dual and null differentiation, so c-kit positivity is not limited to special category of GISTs.

All of the tumors with malignant behavior were large, and had high mitotic activity. Some of them had no necrosis or obvious anaplasia. Although, similar to previous ones, this study showed the importance of mitotic rate and size in the prognosis, a subset of GISTs has unpredictable behavior. None of the currently accepted data for the evaluation of malignancy is definite and enough. Therefore, no GIST should be labeled as unequivocally benign, and all patients with GIST should be carefully followed up for indefinite period of time. Moreover, the diagnosis of GISTs without immunohistochemical studies is incomplete.[11-15] This study also revealed that most of neural and smooth muscle differentiations of GISTs were spindle, therefore, H&E staining does not precisely evaluate the type of differentiation; whereas, previous studies in this regard are conflicting.[7-10]

Conclusion

Diagnosis of Gastrointestinal stromal tumors without immunohistochemistry is not complete especially c-Kit reactivity is very important for treatment modalities. All GISTs should be regarded as potentially malignant tumors and followed up for indefinite period of time.

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