GASTROINTESTINAL STROMAL TUMOURS

Lt Col KAILASH CHAND^{*}, Maj T CHATTERJEE⁺

ABSTRACT

The histogenesis of 10 spindle cell tumors of gastrointestinal tract (GIT) of last three years of our institution was studied, on the basis of immunohistochemical (IHC) staining. The tumours were categorized into benign, borderline malignant and malignant, mainly based on the histologic parameters (HP) such as predominant cell type, cellularity, type of growth pattern, mitotic figures etc. The study included 7 males and 3 females in the age group from 21 to 56 years. 4 tumors were located in stomach and 6 in the small intestine. The initial symptoms included pain abdomen, palpable mass and general weakness. There were 7 benign, 1 borderline and 2 malignant leiomyomatous tumors according to the histologic parameters we followed. Vimentin, desmin, S-100, and non-specific enolase (NSE) were the immunological markers used to identify the cellular lineage. Smooth muscle differentiation was demonstrated by nine (6 benign and 3 malignant) tumours. One male patient (Case 1), originally reported as leiomyoma based on HP, was positive for S-100 and NSE. Another case, a female, categorised as leiomyosarcoma, showed dual differentiation toward myogenic and neural elements on IHC staining (Case 2). IHC study played important role in the identification of cellular differentiation of these tumors as the same was not possible on light microscopic findings alone.

MJAFI 2000; 56 : 40-44

KEY WORDS: Duodenum; Jejunum; Ileum; Gastrointestinal stromal tumor (GIST)-stomach; Soft tissue tumors of GIT.

Introduction

Mazur and Clark were the first to term the non-epithelial tumors of GIT as stromal tumor [1]. These mesenchymal tumors were traditionally regarded as leiomyomatous in origin and their further histologic types were mainly based on the morphologic features on light microscopy [2-4]. Of late, these tumors showed interesting divergent cellular pathways suggestive of smooth muscle, neural and immature mesenchymal differentiations based on the newer diagnostic techniques, such as immunochemistry, and electron microscopy (EM). The histological appearances alone do not reliably reflect the accurate immunophenotypes [5-7]. The classification of Gastrointestinal stromal tumors (GISTs) has been the subject of considerable debate, however based on IHC and EM studies carried by numerous authors, these tumors are divided in four major groups [3,8]. The first two are more common and differentiated groups of tumor, which show cellular differentiation towards either smooth muscles or neural elements. Less commonly, tumors reveal dual differentiation in the third group, and lastly a group of immature mesenchymal tumors lacking differentiation toward either cell types. Some workers use the term GISTs only for undifferentiated stromal tumors of GIT, lacking differentiation towards either cell types [3,7].

With these unsolved aspects of GISTS, we reviewed the histomorphologic features of our cases and studied with immunological markers. Two out of the

Ten cases, which were reported on the histomorphological features, as leiomyoma and leiomyosarcoma revealed neural differentiation in one and dual cellular differentiation in another respectively, on IHC studies. In rest of the cases, the histological diagnosis was consistent with the findings as revealed on IHC stainings.

Material and Methods

It was a retrospective study and we took out all the slides of leiomyomatous tumours of GIT, reported in last three years in our institution. The tumours, which showed no ambiguous histomorphological features of neural or smooth muscles differentiation on light microscopy, were excluded from the study. We identified 10 cases for IHC studies, 7 of which were reported as leiomyoma, 1 borderline malignant and 2 leiomyosarcoma. The haematoxylin and eosin (H&E) stained slides and paraffin embedded blocks of formalin fixed tissue of the tumors were available for review. The tumors were classified as benign, border line malignant or malignant, mainly on the basis of histologic features using parameters such as, predominant cell type, growth pattern, cellularity, nuclear pleomorphism, number of mitotic figures per 50 high power fields, and tumor necrosis etc. Location of the tumors, size and gross morphology, was also kept in mind.

To confirm the histological diagnosis, the paraffin sections of the tumours were studied immunohistochemically to identify the cellular lineage, using markers such as vimentin, desmin, S-100 and NSE. Streptavidin-biotin complex method of IHC staining was used based on the modification of method of Gown et al [21]. Representative deparaffinised sections were rehydrated and treated with 3% hydrogen peroxide in methanol solution, and washed with buffered saline. Antigen retrieval was done by microwaving. Prediluted antibodies (DAKO product) against S-100, desmin vimentin and NSE were used. To check the sensitivity and specificity, nonimmune rabbit serum was used as negative and multi-

^{*} Classified Specialist (Pathology), Military Hospital (CTC) Pune, ⁺ Classified Specialist (Pathology), Base Hospital, Delhi Cantt.

tissue sections as a positive controls.

Results

In the clinicopathologic findings, there were 7 men and 3 women, all in the age groups between 21 to 56 years (mean age 39). All the patients were referred and the tumors were large enough to cause symptoms. The common presenting complaints were vague discomfort or pain abdomen, palpable mass, passing of tarry stools or general body weakness. Ultrasound of abdomen was the most helpful investigation in the localisation of the tumours besides barium meal examination. 4 tumors were located in the stomach and 6 in small intestine. All the tumors were removed surgically except two, both were incidentally malignant and inoperable, however open tissue biopsy was taken, for the purpose of establishing the diagnosis. The tumors ranged from 2.5 to 28 cm in size, except one of the malignant tumor, which had metastasized locally in the loops of ileum, and the exact size of the tumor could not be ascertained. Clinical follow up of the cases revealed that none of the patient with benign lesion progressed further. One of the patients with a malignant tumor died and two lost follow up.

Histologically, six tumors were composed of uniformly spindled cells with abundant cytoplasm, having predominantly fascicular growth pattern and imparted moderate cellularity. There was mild nuclear pleomorphism and mitotic activity was minimal. Skeinoid fibres were seen in less cellular areas and none of these tumors showed areas of necrosis. Higher cellularity was present in two tumors where majority of the cells showed epithelioid cell type, predominantly mixed pattern of growth and no skeinoid fibres. Moderate nuclear pleomorphism and small foci of tumor necrosis were present. Another two tumors revealed spindle cell type with high cellularity, moderate nuclear pleomorphism and more than 5 mitotic figures in 50 high power fields.

IHC studies in all the cases showed 100% positivity to vimentin. Desmin demonstrated positive reaction in only 4 (40%) cases. Two cases are discussed below, one of which stained positively with S-100 and NSE in favour of neural origin of the tumor (Case 1). Another (Case 2) revealed dual cell differentiation on IHC as evident by the positive immunostaining with all the four markers used. The IHC findings of other 8 cases were consistent with the morphological findings of lesions.

Case-1

A 27-year-old male presented with a progressive painless mass in the lower abdomen of three months duration. The patient was a

Fig. 1 : Microphotograph of stromal tumour with neural differentiation (case 1) showing fascicles of uniformly composed spindle cells (scanner view)

known karate player and he had gained 5 kgs weight during the period of illness, inspite of regular exercises. There was history of repeated trauma to the abdomen while playing karate. On physical examination, there was a large smooth surfaced firm mass, palpable in the centre of the abdomen with poorly delineated margins. Other systemic examinations were within normal limits. Routine haematological, biochemical and urinary examinations revealed no significant abnormalities. Radiographs of chest and abdomen showed no significant findings. Ultrasonography of the abdomen revealed, a mass echoic lesion lying anterior to the coils of intestine. FNAC of the mass was inconclusive. A large mass arising from the wall of intestine was removed on exploratory laparotomy. Gross examination of the specimen showed a well-circumscribed sessile tumor, arising from the serosal surface of the small intestine, greyish white colour with glistening smooth surface. It was firm in consistency, weighed 5.7 kg, and measured 28x20x8cm in size.

Microsections of the tumor along with wall of the small intestine revealed the origin of the tumor from muscularis mucosae disrupting the muscle fibres. It was composed of spindle shaped cells, which morphologically resembled smooth muscles but showed tendency toward palisading at places (Figs. 1,2). The microsections were positive for S-100 and NSE and negative for desmin. IHC suggested neural origin of the tumor, probably derived from mesenteric nerve plexus. The patient is asymptomatic for last one year.

Case-2

A 58-year-old lady presented with a large mass in lower half of the abdomen, of 2 1/2 years duration. It was painless to start with but caused discomfort after one year of its onset. She had undergone an abdominal operation in a civil institution for a similar intra-abdominal mass, three years prior to reporting to this hospital. The biopsied tissue was reported as schwannoma. On evaluation, an ill-defined mass was present in the iliac region of abdomen measuring approximately 12 x 10 cm in size. It was firm, immobile, and adherent to the deeper structures. The surface was irregular and nodular at places. Other systemic examinations were within normal limits. Hematological investigations revealed Hb 9g/dL, TLC 8,900 cmm and 79% neutrophils on differential count. Biochemical tests and urinary examination revealed no abnormalities. Abdomen ultrasonography showed 18 x 15 cm echoic mass lesion occupying left side of the abdomen. On exploratory laparotomy, the tumor was found inoperable. It was apparently arising

Fig. 2 : Low power view of Fig. 1 showing bundles of cells and spindling characteristic of the nuclei. Small areas of skeinoid fibres are also seen

Fig. 3 : Microphotograph of stromal tumor with dual differentiation (Case 2) - showing spindle shaped cells with no clear indication towards cellular differentiation

somewhere from the coil of small intestine and adherent at multiple sites to its serosal surfaces and omentum. Exact origin of the tumour could not be ascertained due to jumbled up mass. A small tumor tissue was removed for the tissue diagnosis. Microsections of the tumor showed, spindle shaped cells with pointed ends of the nuclei. Cytoplasm was abundant, eosinophilic and fibrillary at places (Fig 3,4). The tumor on IHC studies revealed, muscle and neural cell differentiation as suggested by strongly positive S-100 and weakly positive NSE, desmin and vimentin.

Discussion

GISTs represent a heterogeneous group of tumors whose classification frequently requires IHC and EM studies. These tumors have shown multidirectional differentiation with immature mesenchymal derivatives in some of the tumors [3-6]. The terms leiomyoma and leiomyosarcoma therefore have been gradually replaced by stromal tumors. GISTs frequently arise from any part of GIT but mainly involve stomach and parts of small intestine. The clinical behaviour of GISTs remains controversial, since most of the workers advocate different criteria for tumors arising from different anatomical sites. Histologic features and prognostic parameters also vary depending upon the site in the GIT [6,8-10].

We classified the tumors as benign, borderline and malignant based on the gross and histological parameters and predicted the outcome reasonably accurately. To differentiate between clinically benign and malignant tumors originating from various sites, several large clinicopathologic studies of GISTs have accepted the contention that tumor size more than 5 cm will have unfavourable prognosis [7,10-12]. The size of the tumor has also been correlated with the histopathological findings in deciding the nature and their metastatic potential [8,9,13]. We considered Appelman criteria [14] of mitotic figures as the single most

Fig. 4 : High power view of Fig. 3 showing spindle shaped cells. Most of the nuclei are having tapering ends, a few showing nuclear pleomorphism in loose myxoid areas between the cells.

important microscopic feature for considering the tumor as malignant and a powerful prognostic indicator. The importance of mitotic figures have been accepted uniformly by various authors to separate benign from malignant lesions, but with variable number. However most workers considered unfavourable outcome of the case if the number of mitotic figures were more than 5 in 50 high power fields [2-7, 16]. Poor prognosis was also predicted, if in addition to the criteria of the tumor size it showed high cellularity, absence of predominant organoid growth pattern and absence of skeinoid fibres on microscopic examination. Features, such as severe nuclear pleomorphism, areas of necrosis and high mitotic figures invariably favoured malignancy [6,7,11,15]. Unfortunately all these conventional features and special stains do not help much in regard to their cellular lineage e.g. tumor occurring in patients with Reckling-hausen's disease, has not necessarily resembled neural fibres and shown pallasading. Some authors included presence or absence of metastasis in the prognosis of clinical behaviour [11].

The tumors apparently showing differentiation towards neural elements and positivity with S-100 and NSE, may have been arising from neural cells of the autonomic nervous system. Such tumors have been designated as gastrointestinal autonomic nerve (GAN) tumors or plexoma/sarcoma [3-6]. GAN tumors are considered malignant as these tumors invariably behave aggressively.

GISTs are well known for their wide variability in the clinical behaviour and difficulty in determining their malignant potential. Majority of the cases occurs in adults but a few have also been reported in children where most of these are malignant [17]. The tumors

are located more commonly in the pars media of stomach followed by antrum and pylorus with or without area of ulceration. In the bowel, these tumors occur anywhere, but these are more commonly found in the ileum. Grossly, the tumors predominantly occur as submucosal rather than subserosal lesions. In large majority of the cases, the tumors are well-circumscribed solitary lesion, but multiple nodular lesions have also been reported [19]. These lesion may be large enough to cause obstruction. The pain abdomen and bleeding remain the commonest clinical presentation [3-5,7,12]. Franquement [12], considered GISTs of small bowel as malignant, or at least of indeterminate malignant potential, if the tumor measured over 5 cm in size, revealed fresh tumor necrosis or extensive haemorrhage related to surgery. Simultaneously it should reveal extreme cellularity, marked atypia and high mitotic figures on microscopy. Metastases take long time to occur in GISTs and the common sites of metastases are lung, liver, peritoneum, and the adjacent structures of abdomen [2,7].

Numerous workers have stressed the importance of IHC in the field of GISTs in their studies. In our study, one tumor (Case 1) categorized as leiomyoma of ileum demonstrated neural differentiation on IHC. Another patient with the tissue diagnosis of schwannoma and history of undergoing small bowel resection (Case 2), was found to have dual cell differentiation on IHC. The line of cell differentiation could only be finalised in these two cases on the basis of IHC. The common immunohistochemical markers used by various workers were vimentin, desmin, alpha smooth muscle actin and muscle specific actin markers (HHF-35) toward myogenic cells, and S-100 and NSE for neural elements [4,5,7,11,12,18]. Pike et al [20] studied GISTs by using various antibodies depending upon the location of tumors in GIT. He suggested GISTs as relatively undifferentiated rather than truly differentiated tumors of the smooth muscle.

Unfortunately, GISTs lack uniformity on IHC finding [3]. A number of antigens are present in cytoplasm and nucleus of the cells, which causes wide variability of results in the tissue sections. Vimentin is not only restricted to mesenchymal tumors but sometimes it also expresses reactivity in tumors of epithelial and neural origin, obviously limiting its diagnostic usefulness. S-100 antigen has a wide range of expression in the tumors derived from chondrocytes, adipocytes, myoepithelial cells and melanocytes besides glial and schwann cells, thereby dilutes credibility of IHC. NSE positivity is also found in majority of neuroectodermal and neuroendocrinal neoplasms including carcinoids

and malignant melanoma. GISTs, often lack desmin, and its positivity does not differ for benign or malignant tumors [2]. The variability in results may be due to the use of different types of fixative and duration of fixation of tissue, poor preservation of the antigen, and non-standardization of preparatory techniques.

In a large series of 584 cases of GISTs, Truong [13] described in details about the low sensitivity and diagnostic utility of desmin. The negative staining may be observed if myofilaments are sparse. The monoclonal antibodies to HHF-35 and smooth muscle actin have been observed by many authors to be more sensitive [4,5,7,18]. Only two of our tumors, were immunoreactive to S-100 and NSE. The sensitivity to S-100 toward neural elements, has been reported as high as 85% [20]. The consensus finding in the IHC staining in various studies has been the frequent vimentin positivity and low sensitivity to desmin. It has been postulated that at birth, vimentin and desmin express equal immunopositivity but as the age progresses, the expression of desmin becomes poor due to sparse myofilaments [14]. It may thus reflect dedifferentiation of the tumor tissue during the process of neoplastic change. It is also known that smooth muscle cells of arteries and veins are vimentin positive but only focally positive or negative for desmin. The reverse is also true with normal smooth muscles of the muscularis mucosa and propria in GIT [15]. It suggests that the origin of GISTs is from smooth muscles of blood vessels rather than muscularis mucosae or propria. IHC studies demonstrate no correlation of the tumor grade with the immunoreactivity [7,16].

The therapeutic approach to GISTs, is usually surgical but the extent of excision varies depending upon the cellular differentiation. The assessment of cellular proliferative activity and DNA aneuploidy status, are another possible prognostic parameters. Although the number of cases in our series is too small to reach to some conclusion, the findings are consistent with the studies revealing the role of IHC studies in cases of GISTs.

REFERENCES

1. Mazur MT, Clark HB. Gastric stromal tumors: Reappraisal of histogenesis. *Am J Surg Pathol* 1983;7:507-19.
2. Evans HL. Smooth muscle tumors of gastrointestinal tract. A study of 56 cases followed for a minimum of 10 years. *Cancer* 1985;56:2242-50.
3. Gastrointestinal tract. In: Rosai J, ed. *Ackerman's surgical pathology*. 8th ed. Mosby-year book. St Louis, Missouri 1996:645-7.
4. Newman PL, Wadden C, Fletcher CD. Gastrointestinal stromal tumors: Correlation of immunophenotype with clinico-

- pathological features. *J Pathol (England)* 1991;164:107-17.
5. Ueyama T, Guo KJ, Hashimoto H, Daimaru Y, Enjoji M. A clinicopathologic and immunohistochemical study of gastrointestinal stromal tumors. *Cancer* 1992;69:947-55.
 6. Brainard JA, Goldblum JR. Stromal tumors of the jejunum and ileum-A clinicopathologic study of 39 cases. *Am J Surg Pathol* 1997;21(4):407-16.
 7. Ma CK, Peralta MN, Amin MB, Linden MD, Dekovich AA, Kubus JJ, Zarbo RJ. Small intestinal stromal tumors-A clinicopathologic study of 20 cases with immunohistochemical assessment of cell differentiation and the prognostic role of proliferation antigens. *Am J Clin Pathol* 1997;108:641-51.
 8. Appelman HD. Mesenchymal tumors of the gastrointestinal tract. In: Ming SC, Goldman H, eds. *Pathology of gastrointestinal tract*. Philadelphia, WB Saunders. 1992:310-50.
 9. Appelman HD. Smooth muscle tumors of gastrointestinal tumors: What we know that Stout did not know. *Am J Surg Pathol* 1986;10(supplII):83-99.
 10. Haque S, Dean PJ. Stromal neoplasms of the rectum and anal canal. *Hum Pathol* 1992;23:762-7.
 11. Goldblum JR, Appelman HD. Stromal tumors of duodenum: A histologic and immuno-histochemical study of 20 cases. *Am J Surg Pathol* 1995;19:71-90.
 12. Franquement D, Frierson H. Muscle differentiation and clinicopathological features of gastrointestinal stromal tumors. *Am J Surg Pathol* 1992;16:947-54.
 13. Tworek JA, Appelman HD, Singleton TR, Greenson JK. Stromal tumors of the jejunum and ileum. *Mod Pathol* 1996;9:379A.
 14. Appelman H, Helwig E. Gastric epitheloid leiomyoma and leiomyosarcoma (leiomyoblastoma). *Cancer* 1997;38:708-28.
 15. Tsutsumi Y, Kubo H. Immunohistochemistry of desmin and vimentin in smooth muscle tumors of the digestive tract. *Acta Pathol Jpn* 1988;38:455-69.
 16. Franquement DW. Differentiation and risk assessment of gastrointestinal stromal tumors. *Am J Clin Pathol* 1995;103:41-7.
 17. Perrson S, Kindblom LG, Angervall L, Tisell LE. Metastasizing gastric epitheloid leiomyosarcoma (leiomyoblastoma) in young individual with long term survival. *Cancer* 1992;2:721-32.
 18. Min KW. Small intestinal stromal tumors with skeinoid fibres: Clinico-pathological, immunological ultrastructural investigations. *Am J Surg Pathol* 1992;16:145-55.
 19. Vallaey JH, Cuvelier CA, Bekaert L, Roels H. Combined leiomyomatosis of the small intestine and colon. *Arch Pathol Lab Med* 1993;116:281-3.
 20. Pike AM, Lloyd RV, Appelman HD. Cell markers in gastrointestinal stromal tumors. *Hum Pathol* 1988;19:830-4.
 21. Gown AM, deWever N, Battifora H. Microwave-based antigenic masking :A revolutionary technique for routine immunohistochemistry. *Appl Immunohistochemistry* 1993; 1: 256-66.