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GASTROINTESTINAL STROMAL TUMOURS AT THE UNIVERSITY OF NIGERIA TEACHING HOSPITAL ENUGU, NIGERIA: AN IMMUNOHISTOCHEMICAL STUDY OF GIT MESENCHYMAL TUMOURS

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ABSTRACT
Mesenchymal tumours of the gastrointestinal tract (GIT) are uncommon. Recent progress in the understanding of the biology and origin of these tumours has led to their reclassification. A new subclass designated Gastrointestinal Stromal Tumours (GIST) is diagnosed based on the presence of a mutational over expression of c-kit protein that is thought to be critical in the pathogenesis of these tumours. This new class of tumours may form the majority of gastrointestinal mesenchymal tumours. Even though the diagnosis of GIST is mainly based on positive staining with CD117, a minority of tumours with histological characteristics of GIST are CD117 negative and are classified as CD117 negative GIST.

In this first review of mesenchymal GIT tumours from Nigeria, we present 11 cases of mesenchymal tumours of the gastrointestinal tract seen within a six-year period at our centre. Immunohistochemistry was performed on 7 of them in which histological appearances suggested GIST. Only two cases had all the criteria defined in the consensus conference on the diagnosis of GIST. Our findings, albeit in a very small sample, contrasts with what obtains in developed countries in the proportion of GIT mesenchymal tumours that are truly GIST. This raises a question to be answered on the true nature and proportion of gastrointestinal strumal tumours among GIT tumours in Nigerian patients.

Key Words: Gastrointestinal, Stromal, Mesenchymal, Immunohistochemistry. *(Accepted 20 August 2009)*

INTRODUCTION
Mesenchymal tumours of the gastrointestinal tract are classified into four main categories based on light and electron microscopy as well as immunohistochemistry. These include those that show smooth muscle differentiation (leiomyoma, leiomyoblastoma, leiomyosarcoma), those that show neural/schwannian differentiation (schwannoma, malignant peripheral nerve tumours [MPNST]), those that appear to differentiate in both directions, and those that show no (null) differentiation at all[1]. Recently, a molecular genetic abnormality resulting in over expression of the tyrosine kinase growth receptor, c-kit has been recognised as vital in the pathogenesis of a significant proportion of these tumours. These have been linked histogenetically to the interstitial cells of Cajal which express c-kit.[2, 3] The importance of detecting this mutation has been greatly emphasized by the finding that a drug STI571, (imatinib [Glivec®, Gleevec®]; Novartis, Basel, Switzerland) which acts to inhibit the c-kit receptor, has a dramatic clinical therapeutic effect on inoperable and metastatic cases of this class of tumours in which the c-kit mutation is present.[4,5]

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This drug which also selectively inhibits other tyrosine kinases like BCR-ABL [chronic myeloid leukaemia] ABL and PDGFR, is more effective and has less severe side effects than standard chemotherapeutic regimen because of the different mechanism of action (specific growth receptor molecule inhibition, rather than general cytotoxicity) [6]. STI571 has therefore heralded a new era of molecular biology based anti-tumour therapy that promises to be revolutionary.

These events led to the consensus conference reclassification of stromal/mesenchymal tumours of the gastrointestinal tract.[7] The new classification separates into a distinct group, those stromal/mesenchymal tumours of the gastrointestinal tract in which a c-kit mutation is detected by immunohistochemical reaction with the CD117 antibody, and these are referred to as *GIST - gastro intestinal stromal tumours*. The diagnostic label *GIST* implies expectation of a response to therapy with STI571 and is hinged on immunohistochemical demonstration of CD117 positivity in stromal/mesenchymal tumours of the gastrointestinal tract[7].

These recent advances have been made based on studies of patients and materials derived from the main populations of the developed nations in

America, Europe and Asia, with little if any contribution from African nations. Considering that molecular genetic alterations in a given tumour may and do differ between ethnic populations, and that drug response and toxicity profile may be affected by the gene's environment and behaviour,[8] we thought it should be important that stromal/mesenchymal tumours of the gastrointestinal tract be similarly studied in African populations. In this communication, we present the first documented immunohistochemical study of mesenchymal tumours of the gastrointestinal tract in patients from Nigeria.

PATIENTS AND METHODS

We reviewed the histology of all tumors of the gastrointestinal tract that were submitted to the Pathology Department of the University of Nigeria Teaching Hospital Enugu, Nigeria from January 2002 to December 2007. All cases of gastrointestinal mesenchymal tumours diagnosed as possible GIST on routine haematoxylin/eosin examination were further subjected to immunohistochemistry. The immunohistochemistry was performed at Harold Wood Hospital UK and read by one of the authors (IUO). It was performed manually using the standard streptavidin-biotin-peroxidase technique and followed the manufacturer's instructions for the antibodies listed in table 1. The antibodies tested included neuron specific enolase (NSE), smooth muscle actin (SMA), desmin, synaptophysin, S100, CD34 and CD117. Antigen retrieval for CD117 was by heating the sections in 0.01M EDTA at pH 8.0 in the microwave oven on high power for 17 minutes, allowing boiling for 5 minutes. For all the other antibodies, sections were heated in 0.01M citrate buffer at pH 6.0 in the microwave oven on high power for a total of 25 minutes, allowing boiling for 10 minutes. The slides were counterstained with Gills hematoxylin.

RESULTS

Ninety six gastrointestinal tumors were received during the period; eleven of them (11.4%) were diagnosed as mesenchymal stromal tumours after routine hematoxylin-eosin processing. Among these eleven cases were one liposarcoma, one fibrosarcoma and nine possible gastrointestinal stromal tumors. The tissue block of two of the possible cases of GIST could not be traced. Immunohistochemistry was done on the remaining seven cases. There were three males and four females with age range of 26 year to 62 years (mean age 43.4 years). Six of the tumours were gastric in origin while one was from the jejunum. The gastric tumours presented with massive upper gastrointestinal haemorrhage in four patients, two of whom had associated abdominal masses, the other two patients presented with abdominal masses only. The jejunal tumour presented with massive lower GI haemorrhage. The duration of symptoms ranged from 6 days to 1 year. Combinations of barium meal and or abdominal ultrasound scan were used to make a preoperative diagnosis of a gastric mass and or ulcer in all the patients (Table 2). All the patients at surgery had exophytic fleshy growths which were still localized to the stomach in three cases. One gastric and the jejunal tumour had infiltrated the surrounding structures while two gastric tumours had distant abdominal metastasis. All the gastric tumours had partial gastrectomy except the two patients with metastatic disease who had wide excision of the primary gastric lesion. Small gut resection with partial cystectomy was done for the jejunal tumour. There was no procedure related mortality.

Immunohistochemistry **(Table 3, Figures1, 2 and 3)**

On immunohistochemistry, tumour cells from two patients (FA and NR) expressed CD34 in a strong and diffuse manner, but CD117 (c-Kit) in a weak and patchy manner. These cases are therefore considered true c-kit positive GIST. Of the five patients whose tumour cells were negative for CD117, two (UP and UT) stained positively for smooth muscle marker, SMA and negatively for NSE and synaptophysin. These two were therefore considered frankly leiomyosarcomas. Among the remaining three patients, two (UL and UK) had tumours that stained positively for NSE but were negative for all other antibodies except CD34 while one was negative for all markers except CD34. These three cases were therefore considered c-kit negative GIST.

Table 1: **Antibodies used in Immunohistochemistry of the Tumours.**

Antibody	Manufacturer	Clone	Dilution
NSE (neuron specific enolase)	DAKO	M0874	1/500
SMA (smooth muscle actin)	Dakocytomation	1A4	1/25
Synaptophysin	Dakocytomation	SY38	1/5
CD34	Novocastra	QBEND/10	1/20
CD117	Dakocytomation	Rabbit Polyclonal	1/400

Table 2: **Diagnostic and Operative Findings.**

	Diagnostic tool	Op findings	Procedure done
FA	Barium meal: mass at lesser curvature with ulcer crater	Gastric exophytic tumour with mucosal protrusion and ulceration	Partial gastrectomy, Bilroth I
IC	Barium meal: feeling defect in stomach	10x14cm lesser curve gastric tumour with 2 deeply ulcerated areas	4/5 distal gastrectomy , Bil I
UL	Ultrasound: gastric mass, Barium meal: no mass or ulcers in stomach, OGD: inflammation and heamatoma in antrum	20 x20cm gastric corpus tumor, adherent to jejunum & transverse colon.	Hoffmeister partial gastrectomy, transverse colectomy
UP	Barium enema: normal, Ultrasound: echogenic mass 10x12cm related to the posterior wall of urinary bladder	Fleshy exophytic jejunal mass, adherent to urinary bladder,	Small intestinal resection with partial excision of blader wall
UT	Ultrasound: retroperitoneal cystic mass possibly pancreatic pseudo cyst	huge cystic mass with a solid mushroom like componenent originating from gastric fundus and cardia.	wide excision
NR	Ultrasound: gastric mass; Barium meal: filling defect in cardia and lesser curvature of stomach	18 x26cm mass in cardia, fundus and body of stomach	proximal gastrectomy
UK	Ultrasound scan = malignant mass in retrogastric space with liver metastasis.	10 cm gastric tumour in the retrogastric area, adherent to tail of pancreas, spleen, solitary hepatic nodule	Wedge resection of gastric tumour, splenectomy, distal pancreatectomy and liver metastatectomy

Table 3: **Pathology**

Patient's initials	FA	IC	UL	UP	UT	NR	UK
Initial H/E report	GIST, intermediate grade with smooth muscle differentiation	Borderline GIST, smooth muscle type .	malignant GIST, smooth muscle type	Borderline GIST	Leiomyo sarcoma with metastasis	Moderately well differentiated leiomyo sarcoma	Malignant GIST, metastatic
NSE	+ve	-ve	+ve	-ve	Not done	Not done-	very weak & focal
SMA	-ve	-ve	-ve	+ve	+ve	-ve	-ve
Synaptophysin	+ve	-ve	-ve	-ve	-ve	Not done	-ve
S100	Not done	Not done	Not done	Not done	Not done	-ve	-ve
CD34	+ve	+ve	+ve	-ve	+ve	+ve	+ve
CD117	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Final diagnosis	ckit +ve GIST	CD117 -ve GI mesenchymal tumour	CD117 -ve GI mesenchymal tumour	Leiomyo sarcoma	Leiomyo sarcoma	ckit +ve GIST	CD117 -ve GI mesenchymal tumor, null differentiation

Figure 1: **Case FA; immunohistochemistry with CD34 showing strong positive staining with tumour. Note muscularis mucosa is negative. Magnification x10.**

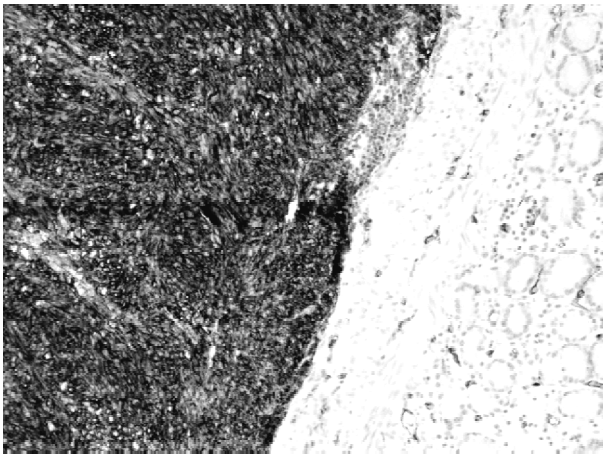


Figure 2: **Case FA; immunohistochemistry with CD117 (c-kit) showing positive staining of tumour. Magnification x4.**

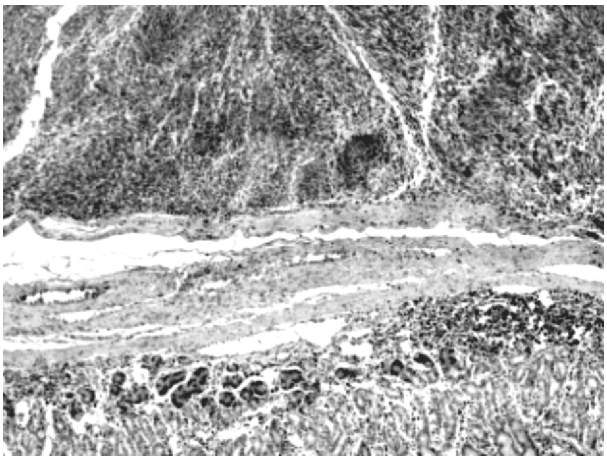
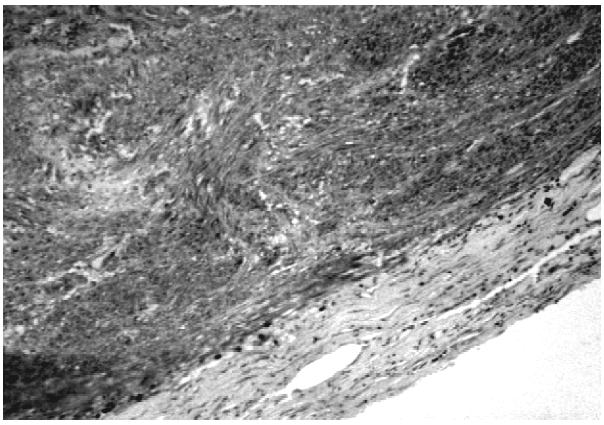


Figure 3: **Case UL: Immunohistochemistry with CD117 (c-kit) showing negative tumour reaction (note positive internal control with mucosal mast cells).**



DISCUSSION

Most mesenchymal tumours of the gastrointestinal tract are said to be either GIST or of smooth muscle type [9]. In eleven gastrointestinal mesenchymal tumours seen in our centre, two were true CD 117 positive GIST while two cases were histologically and immunohistochemically diagnosed as smooth muscle tumours. Three other patients were unclassified and are considered C-kit negative GIST. Schwanoma, a rare spindle cell tumour of the stomach may grow to a large size and therefore is a differential here. However apart from the absence of any distinct histologic features to suggest this diagnosis, gastric schwannomas rarely ulcerate the mucosa and are infrequently positive with CD34 as seen in these three cases. The other rare possibility of fibromatosis is also excluded not only by the gross appearance of the tumours but also by the negative reaction for SMA and positive reaction for CD34 in the three unclassified cases (IC, UL and UK). In the absence of reliable immunohistochemical/histologic features of smooth muscle or neural differentiation in the three cases (IC, UL and UK), it seems reasonable to classify them as probable GISTs especially with their positivity with Cd34.

Only two of our cases fulfilled the immunohistochemical diagnostic criteria set out in the consensus approach reached at the US National Institutes of Health GIST workshop of April 2001[7]. According to that consensus, a rare GIST may be CD117 negative in the following circumstances.

1. An immunohistochemically inert sample possibly as a result of fixation or other processing artefact. The strong reaction with CD34 in our cases and other positive internal control parameters suggests that this does not apply.
2. Sampling error such as may be seen in needle biopsies, would also not apply in our cases.
3. Cessation of KIT expression due to some of clonal evolution perhaps following STI571 therapy. None of our patients has received the drug.
4. A small percentage (<2%) that lack either KIT mutations and/or KIT over expression.

It would appear that three of our cases belong to this last category of true CD117-negative GIST. This of course raises the question of the true proportion of CD117-negative GIST and whether this varies with the environment in which the study was done. Our numbers are too small for a generalization to be made but it raises a question whether the proportion of CD117-negative GIST from our population might be higher than the 2% recognized in the consensus document. Indeed some authors have questioned the position that makes CD117 positivity an absolute requirement for the diagnosis of GIST, especially

considering that this does not necessarily indicate that a mutation of the gene is present[10]. Conversely a KIT mutation may exist in the absence of the immunohistochemically detectable marker.

In summary we have analysed eleven cases of gastrointestinal mesenchymal tumours seen in our hospital over a six year period.

Immunohistochemistry was performed in seven of nine cases in which histology suggested GIST. Only two cases had all the criteria defined in the consensus conference on the diagnosis of GIST. Our findings raise some questions on the true nature and proportion of GIT mesenchymal tumours in our environment that are GIST. A determination of this is very crucial in knowing the usefulness of the new targeted treatments for gastrointestinal strumal tumours in our environment. Since this category of GIT tumours is uncommon, more collaborative studies between institutions from different parts of Nigeria and Africa are needed in order to meaningfully contribute to this area of tumour pathology that promises to significantly change the way this group of malignancies are managed.

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