RESEARCH ARTICLE

Histomorphology and Immunohistochemistry of Gastrointestinal Stromal Tumors in a Malaysian Population

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Abstract

Purpose: To study histomorphological and immunohistochemical patterns of gastro-intestinal stromal tumours (GISTs) in Malaysia. Materials and Methods: A total of 29 GIST cases from Hospital Tuanku Ja'afar, Seremban ,were studied retrospectively over a period of 10 years from January 2002 to December 2011. Patient demographic data like age, sex and etnicity were collected. Tumour characteristics like site, maximum dimension and specimen type were analysed. Evaluation was according to established criteria into very low, low, intermediate and high-risk categories. Immunohistochemical characteristics were also analysed. Results: The mean age of patients was 59.7 years. Males (59%) were found to be more commonly affected than females (41%). The Chinese (45%) were commonly affected than Malays (41%), and Indians (10%). The most common symptom was pain in the abdomen (13.8%). More than half of the cases were seen in stomach (53%). The tumour size ranged from 1.5 cm to 17 cm with a mean of 6.94cm. Microscopic findings revealed that the spindle cell type was the most common (76%). It was observed that the majority of the cases (48%) were categorised in the intermediate risk group. Immunohistochemical staining showed positivity for CD117 (78.6%), CD34 (71.4%), vimentin (86.2%), S-100 (27.6%), SMA (35.7%), PKC THETA (46.4%) and PDGRFA (67.9%).

Keywords: Gastrointestinal stromal tumor - immunohistochemistry - risk categories - spindle cell - CD 117

Asian Pac J Cancer Prev, **17 (6)**, 2795-2799

Introduction

Gastrointestinal stromal tumours (GIST) is the most common form of the gastrointestinal sarcomas accounting for 2% of all gastrointestinal neoplasms (Jiang et al., 2016). GIST can occur in any part of the GI tract, but Stomach being the most common location (Demetri GD et al., 2007). Most of the GISTs have the potential to turn malignant, however a subset of these lesions particularly small and asymptomatic are benign in nature (Daniel R et al., 2013). The histogenesis of GIST has been in debate continuously and is now believed that the gastrointestinal pacemaker cells, the Interstitial Cells of Cajal (ICC) plays a central role in their pathogenesis (Demetri et al., 2007). Immunohistochemical studies have helped greatly and are now compulsory in diagnosis of GIST and to differentiate them from other mesenchymal tumors of the gastrointestinal tract. As a result of major recent advances in understanding the biology of GISTs, these tumours have become the focus of considerable attention by clinicians and have resulted in a lot of dynamicity with regard to classification, lines of differentiation and diagnosis. To identify and appropriately manage the GISTs at an early stage, it needs a systematic investigative approach which in-turn depends on the various characteristics of the Tumor and its subtypes (Daniel et al., 2013).

We retrospectively studied 29 cases of GISTs for the histomorphological and immunohistochemical characteristics of GISTs. The study also aimed to sub stratify the GISTs according to its biological behaviour.

Materials and Methods

Twenty nine GIST cases were selected retrospectively over a period of 10 years from January 2002 to December 2011 from the archives of the pathology department, Hospital Tuanku Ja'afar (HTJ), Seremban. After obtaining approval form the ethic committee of International medical university and Blanket consent from the Hospital Tuanku Ja'afar, the demographic data like age, sex, race of patient and tumour characteristics like specimen type, tumour site, maximum tumour dimension etc were

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collected. Furthermore, wherever possible clinical and operative data were retrieved.

The Hematoxylin & Eosin slides from each case were observed by two authors (pathologists) separately for cell type, cellularity, nucleoli, mitotic figures, growth pattern, nuclear pleomorphism, mucosal infiltration, skeinoid fibres and coagulative necrosis.

Cell type was categorised as predominantly spindle or epithelioid (>75% of the tumour cells). Cellularity of the tumour was judged to be low, moderate or high. Nucleoli were judged to be indistinct or prominent. Mitotic figures were counted by examining 50 consecutive high power fields. Mucosal infiltration of invasion was defined as tumour cells breaching the muscularis mucosae and extending into the lamina propria.Tumours were evaluated according to the maximum tumour dimension and mitotic counts and assessed for risk behaviour according to the criteria laid down by Fletcher CDM et al. (2002) into very low, low, intermediate and high-risk categories.

The Allred scoring system for IHC staining in tumour cells was implemented to allow a qualitative and a semi-quantitative analysis on the IHC stained slides. The two parameters of interest were staining intensity (SI) and staining density (SD). Staining intensity (SI) was scored according to the following scale: no visible staining = 0, weak staining = 1+, moderate staining= 2+ and intense staining = 3+. For staining density (SD), the total number of cells with positive staining for each antibody was semi-quantified into five main categories based on the percentage of cells being stained positive: 0 = no cells stained positive, 1 ≤10% of the cells stained positive, 2 = 10-50 % of the cells stained positive, 3=50 -90% of the cells stained positive and 4 ≥90% of the cells stained positive.

Ethics: The study is approved by International medical university joint committee for ethics and national medical research & ethics committee (MREC). The study is registered under national Malaysian research register (NMRR), NMRR no: 11-508-8940.

Results

Ages of the patients ranged from 22 years to 84 years with a mean age of 59.7 years. The study group comprises of 17 men (59%) and 12 women (41%). The Chinese (13 cases, 45%) were commonly affected followed closely by the Malays (12 cases, 41%), the Indians (3 cases, 10%) and the other races (1 case, 4%).

The presenting complaints of the patients were available in 7 (24.1%) cases. The most common symptom was pain abdomen (4 cases, 13.8%) and melena, (4 cases, 13.8%). Other non-specific symptoms include loss of weight (1 case, 3.4%), loss of appetite (1 case, 3.4%), hemaetemasis (1 case, 3.4%) and palpable mass in the abdomen (1 case, 3.4%).

Tumour Characteristics: The maximum number of cases were tumours belonging to the stomach (15 cases, 53%). 9 cases (31%) were from the small intestine including 1 case in the Meckel's Diverticulum. 3 cases (10%) were from other site including the rectum, omentum and pelvis. 2 cases (7%) site of origin was not known.

The tumour site was further analysed according to age groups and it was found that GISTs arising in the stomach were most commonly seen in 60-69 years old age group with 6 cases out of 15 gastric GISTs cases. Tumours arising from the small intestine were most commonly seen in the 50-59 years old age group with 3 cases out of 9 small intestinal GISTs cases.

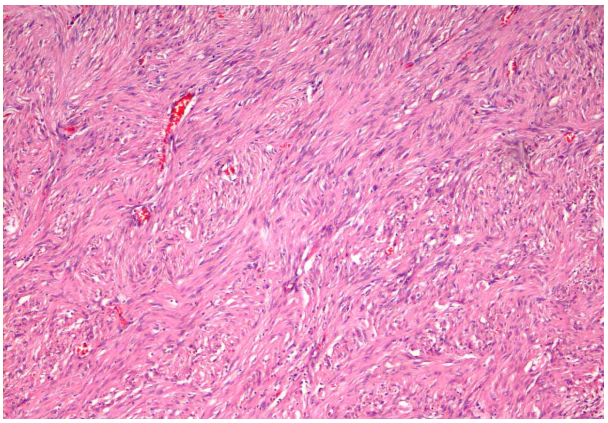

Figure 1. Microscopic Appearance of Spindle Cell Variant of GIST (H&E 200X)

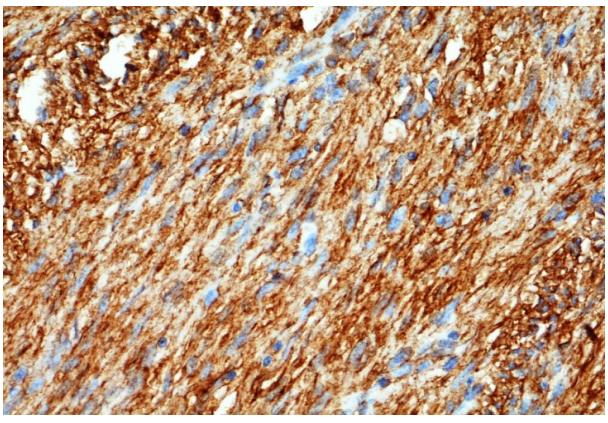

Figure 2. Positive Immunohistochemistry for CD117 (400 X)

Table 1. Comparison of Age, Tumour size and Mitotic count between Groups 1 and 2

Parameters	Group 1 (n=6) (Very low to low risk group) Mean Value	Group 2 (n=19) (Intermediate to high risk group) Mean Value	P value
Age (years)	55.3	63.2	NS (P = 0.2012)
Tumour Size (cm)	3.3	8.1	P = 0.0057
Mitotic Count (per 50 HPFs)	1.5	6.1	P = 0.0414

NS = Not Significant, P > 0.05

Table 2. Immunohistochemical Staining Patterns

Antigen (type)	No. of Cases Positive (%)	Mean Intensity Score	Mean Density Score
CD117 (c-Kit)	22 (78.6)	2.18	2.93
CD34	20 (71.4)	1.79	2.57
Vimentin	25 (86.2)	2.17	3.21
S-100	8 (27.6)	0.52	0.69
SMA	10 (35.7)	0.68	0.82
PKC theta	13 (46.4)	0.93	1.21
PDGRFA	19 (67.9)	1.39	2.04

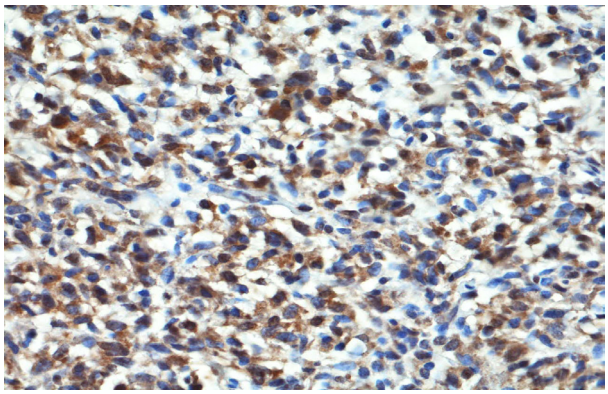

Figure 3. Shows Positive Immunohistochemistry for PDGFR (400 X)

The tumour size ranged from 1.5 cm to 17 cm with a mean tumour size of 6.94cm and it was observed that the gastric GISTs were relatively larger in size as compared to the small intestinal GISTs. Three of the stomach GISTs cases have tumour sizes more than 10 cm .

Microscopy revealed that the spindle cell type (Figure 1) was the most common and this was found in 22 cases (76%), followed by the mixed cell type in 6 cases (21%) and the epithelioid cell type in 1 case (3%). The mitotic count ranged from 0 to 41 per 50 HPFs with a mean count of 5.2 per 50 HPFs.

In the study, 17 cases (59%) were of moderate cellularity and 12 cases (41%) were of high cellularity.19 cases (66%) had indistinct nucleoli whereas 10 cases (34%) had prominent nucleoli. Coagulative necrosis was found in 16 cases (55%). Haemorrhage was reported in 18 cases (62%). Skeinoid fibres were found in 6 cases (21%) where 23 cases (79%) were not found to have skeinoid fibres. Loose myxoid stroma was found in 23 cases (79%) whereas 6 cases (21%) were not found to have loose myxoid stroma.

In this study, the majority of the cases (26 cases, 89%) were moderately differentiated, followed by poorly differentiated with 2 cases (7%) and well differentiated with 1 case (4%).

Risk Categorisation of Tumours

The tumours were evaluated according to the maximum tumour dimension and mitotic counts and assessed for risk behaviour according to the criteria laid down by Fletcher CDM et al. (2002) into very low, low, intermediate and high-risk categories. It was observed that the majority of the cases (12 cases, 48%) were categorised in the intermediate risk group, followed by high risk group with 7 cases (28%), low risk group with 5 cases (20%) and very low risk group with 1 case (4%). 4 cases were unable to be categorised because they were small biopsy cases and therefore, the maximum tumour dimension was not available.

Application of Fletcher's Criteria on Risk Categorisation of GIST

In order to apply the criteria laid down by Fletcher CDM et al. (2002) for risk categorisation of GISTs, the very low and low risk groups were reclassified into Group 1 and the intermediate and high-risk groups were reclassified into Group 2. A simple unpaired t-test was utilised to compare the two group based on three parameters (age, tumour size and mitotic figures) with a p-value of less than 0.05 being statistically significant. This comparison is illustrated in Table 1.

There was no significant difference between the two groups for age. However, tumour size and mitotic figures were significantly different between the two main groups. Therefore, confirming the validity of Fletcher's criteria for risk stratification of GISTs which states that tumour size and the mitotic count are significantly correlated with the risk level of the tumour.

Immunohistochemical staining was positive for CD117 (Figure 2) in 22 cases (78.6%), CD34 in 20 cases (71.4%), Vimentin in 25 cases (86.2%), S-100 in 8 cases (27.6%), SMA in 10 cases (35.7%), PKC theta in 13 cases (46.4%) and PDGRFA (Figure 3) in 19 cases (67.9%).

According to Allred D et al. (1998), the Allred scoring system for IHC staining was implemented and the mean intensity and mean density score for each IHC marker were calculated and tabulated in Table 2. It was observed that CD117 had the highest mean intensity score with a score of 2.18 followed closely by Vimentin with a score of 2.17. Vimentin on the other hand had the highest mean density score with a score of 3.21 followed by CD117 with a score of 2.93.

Discussion

GIST forms an important differential diagnosis while evaluating the Subepithelial tumors (SETs) for the fact most of the SETs are detected while patients undergo screening for cancers and are usually benign and GIST being malignant demands the accurate evaluation of the lesions and final diagnosis (Daniel et al., 2013). In the current study we attempted to analyse the clinipathological characteristics of GISTs in Malaysian population.

GIST is predominantly seen occurring in old age. In our study the mean age of the patients was 59.7 years which was supported by the observations of Miettinen et al. (1999), Na Kang et al. (2010), Steigen et al. (2008) and Khoo et al. (2005). Males (59%) were found to be more commonly affected than females (41%). This observation was in agreement with that of DeMatteo et al. (2002) and Hasegawa et al. (2002). However, in three other series (Mieteeinen et al., 1999; Khoo et al., 2005; Steigen et al., 2008), GISTs showed an equal sex incidence. On the contrary, Na Kang et al. (2010) had reported a higher incidence in females compared to males.

This study group showed that the Chinese (45%) and Malays (41%) were more commonly affected compared to the Indians (10%) and other race (4%). However, observation made by Khoo JJ (2005) found that Malays (84.6%) were more commonly affected than the Chinese (15.4%) with no records of Indian and other race patients.

According to Miettinen et al (1999), the most common site for GISTs was in the stomach (60% to 70%), followed by small intestine (25% to 35%), colon and rectum (5%) and oesophagus (<2%). In this study, the maximum number of cases was tumours belonging to the stomach (15 cases, 53%). 9 cases (31%) were from the small intestine, 3 cases (10%) were from other sites. This was in concordance with observations made in three other series (Khoo et al., 2005; Steigen et al., 2008; Na Kang et al., 2010). The tumour size ranged from 1.5 cm to 17 cm with a mean tumour size of 6.94cm. This observation was in agreement with that of Kim et al. (2005) who reported a mean size of 6.1cm. However, Steigen et al. (2008) had reported a higher mean tumour size of 8.9cm.

Spindle cell type is the most common type of the GIST, which is supported by the present study with 22 cases (76%) of spindle cell type. This was in concordance with observations by Steigen et al. (2008) and Kim et al. (2005).

Various clinical and pathologic parameters have been studied to predict the biological behaviour of GISTs, but with varying results. Age and sex have not been found to show any correlation with the malignant potential of GISTs (Koo et al., 2005). However, some studies revealed that tumours in the oesophagus had a benign behaviour while those in the small intestine exhibited recurrent and metastatic behaviour (Miettinen et al., 2006). This study was unable to dispute or support these findings probably because of the small sample size of tumours in each location and also there were no oesophageal GISTs in this study.

However, in this study, tumour size was found to be significantly correlated to the risk level of the tumour. This was in concordance with the observations by Khoo et al. (2005). Furthermore, a study of 171 GISTs patients conducted by Hasegawa et al. (2002) found that tumours measuring more than 10 cm were associated with a poor clinical outcome.

In addition, mitotic count has been found to be the most reliable predictor of malignant potential of GISTs (Amin et al., 1993). Usually, a cut-off mitotic index of >5/50 HPF was utilised by most literature for distinguishing benign and malignant GISTs. (Amin et al.,1993; Miettinen et al., 2002). In this study, it was found that mitotic figures were significantly correlated to the risk level of the tumour. Therefore, this study supports the contention that mitotic count was predictive of malignant potential in GISTs.

In the present study CD117 (c-Kit) was positive in 78.6%, which is in concordance with the observations of Na Kang et al. (2010), Steigen et al (2008) and Kim et al (2005). CD117 positivity was high in the stomach (86.7%) and in the small intestine (72.7%). This finding correlated with the observations of Kim et al. (2005).

According to Miettinen et al. (2002), CD 34 positivity is seen more in gastric GISTs in comparison to non-gastric GISTs, whereas SMA was more commonly expressed in the non-gastric GISTs, particular small intestinal GISTs. In this study, CD34 positivity was higher in the stomach lesions (80.0%) as compared to the non-stomach lesions (63.6%) which is in agreement with Miettinen et al (2002). However, SMA positivity was also expressed more commonly in the gastric tumours (40.0%) compared to the non-gastric tumours (27.3%) which was not in accordance with the observations of Miettinen et al. (2002). S-100 reactivity was observed to be present more frequently in the non-stomach lesions as compared to the stomach lesions. This observation was in agreement with Khoo et al. (2005).

In addition our study included PKC theta which was positive in 46.4% and PDGRFA in 67.9% of cases.

In conclusion, Stromal tumours arising from the wall of the gastrointestinal tract have produced a massive amount of enthusiasm and interest in recent years. As a result of major recent advances in understanding the biology of GISTs, these tumours have become the focus of considerable attention and have resulted in a lot of dynamicity with regard to classification, lines of differentiation and prognostication. However, the GISTs in South-East Asia and in more particular, Malaysia, are still relatively unexplored.

The study focused on finding out the histomorphology and immunohistochemical characteristics of GISTs in a particular subset of the Malaysian population and correlate both the histomorphological and immunohistochemical patterns to acquire a better understanding of its biological behaviour. To conclude our study emphasised that histomorphology combined with immunohistochemistry can greatly enhance accuracy of diagnosis of GISTs and could be used as prognostic markers.

For further study, there need to be a continuation with the follow-up of GISTs patients and completion of clinical data on recurrence, metastasis and survival. Apart from that, the detection of other more sensitive and specific histologic and immunohistochemical markers that correlate with the prognosis should be looked into further. Moreover, genetic studies on KIT and PDGRFA mutations particularly in KIT-negative GISTs should be another dimension to be explored.

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