ORIGINAL ARTICLES

FREQUENCY OF LOSS OF SUCCINATE DEHYDROGENASE-B IN GASTROINTESTINAL STROMAL TUMORS: SHAUKAT KHANUM HOSPITAL EXPERIENCE

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ABSTRACT

Objective: To assess the frequency of loss of expression of succinate dehydrogenase-B (SDH-B) in gastrointestinal stromal tumors (GISTS) of our population through immunohistochemistry.

Study Design: Descriptive study.

Place and Duration of Study: Department of Pathology, Shaukat Khanum Hospital & Research Centre, Lahore for a duration of 6 months, Apr 2015 to Sep 2015.

Patients and Methods: A total of 63 diagnosed cases of GISTS suitable by inclusion criteria were included in the study. The blocks of already diagnosed cases was reviewed by 2 pathologists and after confirmation SDH-B immunohistochemical stain was applied with normal tissue as its control. Lack of staining in tumor was interpreted as "Loss of expression".

Results: Mean age of our population was n=44.83 ± 17.76 years. The most common tumor site was small intestine n=23 (63.5%) cases, followed by stomach, n=15 cases. CD117 was positive in all the tumors. Only n=3 tumors were found to be SDH-B deficient. All of these tumors involved the stomach and had a median age of 20 years. All of these tumors had an epithelioid morphology.

Conclusion: Immunohistochemistry was found reliable and cost-effective method for detecting mutation in SDH gene. Based on findings of this study it is recommended that all gastric GISTs below the age of 30 years with epithelioid morphology should be tested for SDH deficiency.

Keywords: Gastrointestinal stromal tumors, Immunohistochemistry, SDH-B.

INTRODUCTION

Gastrointestinal stromal tumors (GIST) is the most common mesenchymal tumor of the gastro-intestinal tract comprising 11-19.6 cases per million. The adult form of GIST mainly presents in the sixth decade of life and shows no gender predilection. Although, about one fifth of the tumors are asymptomatic, other tumors present with abdominal pain, gastrointestinal bleeding and symptoms of obstruction[1]. Stomach is the most common location followed by small bowel. It is mainly a submucosal and mural lesion. Surgical excision is the treatment of choice for most tumors[2]. In unresectable and metastatic cases targeted drug Imatinib is used, which is a tyrosine kinase inhibitor (TKI)[3]. Most common type of molecular alteration in GIST is c-Kit mutation followed by platelet derived growth factor receptor (PDGFR) mutations which make up a combined 95% of the tumors. The rest of the 5% of the tumors were previously thought to be wild type tumors, but now they include tumors with mutations in succinate dehydrogenase-B (SDH-B) and BRAF.

SDH-B deficient tumors include Pediatric type GISTS and those associated with Carney Stratakis syndrome[4]. Succinate dehydrogenase consists of four protein subunits: SDHA to SDHD that are localized in the inner mito-chondrial membrane and act at the interphase of the tricarboxylic acid cycle and electron transport chain. Loss of function of SDHB, SDHC or SDHD is central to the pathogenesis of these tumors. These tumors comprise 7.5% of all gastrointestinal stromal tumors and include a group of children, young adults and a small

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Received: 10 Oct 2017; revised received: 27 Oct 2017; accepted: 17 Nov 2017

number of older individuals. This variant only occurs in the stomach and are commonly multi-focal with predominant epithelioid morphology[5].

Deficiency of succinate dehydrogenase can be detected by either molecular methods or by immunohistochemistry. Molecular methods involve laser microdissection and Sanger sequencing. Antibody to succinate dehydro-genase, especially to SDH-B, has shown good correlation with molecular analysis[5].

Normal tissue shows granular cytoplasmic staining of SDHB reflecting the presence of this enzyme in the mitochondria. The SDH deficient cases show loss of expression and this correlates with mutation in genes encoding this protein.

The purpose of this study is to assess the frequency of SDHB deficient GISTS in our cases through immunohistochemistry. These tumors show poor response to Imatinib and behave aggressively with propensity for distant and nodal metastases. Therefore, identification of this type of GIST prompts appropriate measures for management.

MATERIAL AND METHODS

This was an observational study (descriptive study) carried out in the department of Pathology, Shaukat Khanum Hospital and Research centre, Lahore, Pakistan from April 2015 to September 2015. Total number of 63 diagnosed cases of GIST were included through purposive non-probability sampling technique throughout the years 2011 to 2014. Both excison and incision biopsies were included. Reports and slides of all these cases were retrieved. The sample population included patients of all ages, gender, tumor size, tumor site and histological subtypes of GIST. Our exclusion criteria included unfixed or necrotic tissue, history of previous chemotherapy or recurrent tumors.

Table-I: Clinico-pathological characteristics of patients included in the study.

Site	No. of cases	Gender		Histological subtypes			Mean size (cm)**	Mean no of mitosis (per 20HPF)	Risk stratification**			Immuno-histochemical markers Positivity (%)			
		Male	Female	Spindle cell	Epitheloid type	Mixed			Low risk	Moderate risk	High risk	CD117 (n=63)	DOG 1 (n=19)	CD 34 (n=11)	SDHB* (n=63)
Stomach	15	9 (60%)	6 (40%)	12 (80%)	3 (15%)	0	8.9 ± 5.1	7.1 ± 9.3	6	2	4	100%	80%	50%	20%
Small bowel	23	15 (65.2%)	8 (34.8%)	19 (82.6%)	1 (4.3%)	3 (13.04%)	9.8 ± 2.9	6.1 ± 5.2	1	2	13	100%	100%	75%	0%
Large bowel	4	3 (75%)	1 (25%)	4 (100%)	0	0	11.7 ± 3.4	29.8 ± 26.7	0	0	4	100%	100%	-	0
Abdomen (NOS)	21	13 (61.9%)	8 (38.1%)	18 (85.7%)	2 (9.5%)	1 (4.7%)	13.7 ± 5.9	15.4 ± 16.6	1	0	10	100%	90%	50%	0%

*SDHB Percentage is expressed for loss of expression, **Risk stratification and mean size was only done on excision biopsies

The slides were reviewed for diagnosis by two histopathologists with special interest in GI pathology. Parameters including histological subtype, number of mitosis per 20/HPF and risk assessment were reviewed. Immunohistochemical expression of diagnostic markers of GIST CD34, CD117 and DOG-1 were reassessed and documented. SDHB (concentrated rabbit monoclonal antibody, clone EP288 of abcam) immunohistochemical stain was applied to all the blocks with a dilution of 1:20. Non-neoplastic tissue in the blocks was used as internal positive control. Lack of staining in tumor was interpreted as "Loss of expression". The analysis was done through SPSS 22.0. Mean ± S.D was calculated for quantitative variables like age, greatest tumor dimension (T) and mitosis. Frequencies assessed for qualitative variables like gender, risk assessment, and expression of Immunohisto-chemical markers including SDHB.

Table-II: Clinicopathological features of SDH-B deficient GISTS.

Mean age	22.67 ± 6.4 years
Median age	20 years
Mean size	8.0 ± 6.4 cm
Spindle cell type	0
Epithelioid cell type	3
Site	Stomach (3 out of 3)
Risk assessment	Low risk (n=1), Moderate risk (n=2), High risk (n=0)

Table-III: Important clinical and pathological features of SDHB deficient cases in study.

Case no.	Age	Gender	Site	Type of Biopsy	Histological type	Size (cm)	Mitosis	Risk stratification	Margins
1	20	Male	Stomach (Not specified)	Excision (Fragmented)	Epithelioid	15	4	Moderate risk	Not assessable
2	30	Male	Antrum	Excision	Epithelioid	3.5	8	Moderate risk	Negative
3	18	Female	Lesser curvature	Excision	Epithelioid	5.5	4	Low risk	Negative

RESULTS

Mean age of the population was n=44.83 ± 17.76 years ranging from 17 years to 90 years. There were n=40 (63.5%) males and n=23 (36.5%) females. There were n=40 excision biopsies and n=23 incision biopsies. The most common site of tumor in our study was small intestine n=23 (36.5%) cases followed by stomach, n=15 (23.8%) cases and large bowel, n=4 (6.3%) cases and n=21 cases (33.33%) were extra gastrointestinal. Mean size of tumor was 10.74 ± 4.9 cm. Most tumors were spindle cell type n=53 (84.1%) followed by pure epithelioid type n=5 (7.9%) and mixed spindle and epithelioid type, n=4 (6.3%). The mean number of mitoses was 11.13 ± 14.13 per 20/HPF. Risk stratification was only carried out in the excision biopsies (n=40) or in few cases of incision biopsies (n=3) where radiological information was available. Majority of the tumors were included in the high risk category (n=31, 72%). N=4 cases (9.3%) belonged to intermediate type and n=8 (18.6%) cases represented the low risk category. Breakdown of clinicopathological features is presented in table-I. All the tumors (n=63) were positive for CD 117. DOG 1 was performed in n=19 cases, of which 17 were positive. While, CD34 was performed on only n=11 of the cases, and seven of them were positive. There was loss of expression of SDHB in n=3 cases (4.7%). Clinicopathological characteristics of SDHB deficient GISTs are summarized in table-II, clinical and pathological features of SDHB ar summarized in table-III.

DISCUSSION

GISTS are the most common mesenchymal tumors of the gastrointestinal tract[1]. GISTs

occur throughout the tubular gut as well as in omentum, mesentry, pelvis and retroperitoneum. GIST usually present as solitary, rounded lobular masses in the muscularis propria or the submucosa (fig-1). They have a firm gray white cut surface. Microscopically, GIST can show either spindle cell morphology or epithelioid cell morphology. The spindle cell type which comprises of the majority of tumors has uniform pale eosinophilic cytoplasm, oval to short spindled nuclei, paranuclear vacuoles and skeinoid fibers (fig-2). About third of the tumors have epithelioid morphology with polygonal cells, round to oval nuclei and cytoplasmic retraction[2]. About 7-10% of GISTs have loss of function of the succinate dehydrogenase complex on inner mitochondrial membrane. SDHB deficiency is identified in many tumors including renal tumors, paragangliomas and pheochromocytomas. The current study had a sample population of n=63 cases. The median age of our population was 40 years which is far less from the classic study by Miettinen *et al*, who reported a median age of 63 years[6], but closer to another Pakistani study that reported a mean age of 51 years[11]. Both these studies showed male predominance similar to the current study. Spindle cell histology was also the most frequent type in the study by Din *et al*. The same study also showed a large number of high risk cases in stomach and small intestine[7].

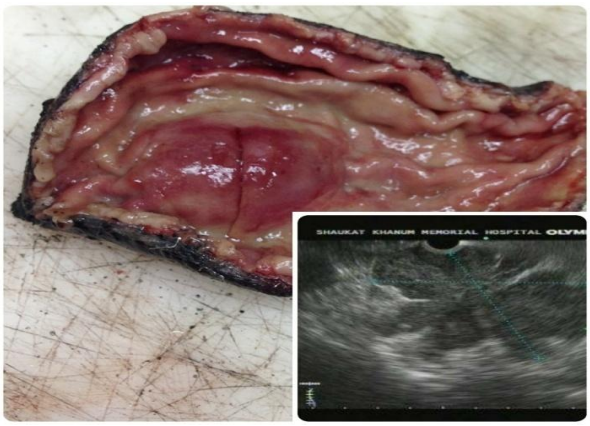

Figure-1: Gross appearance of GIST. There is a submucosal polypoidal lesion showing surface ulceration. Inset: Ultrasonography shows a hypoechoic rounded soft tissue mass, arising from the wall of stomachwith an endoluminal and exophytic growth.

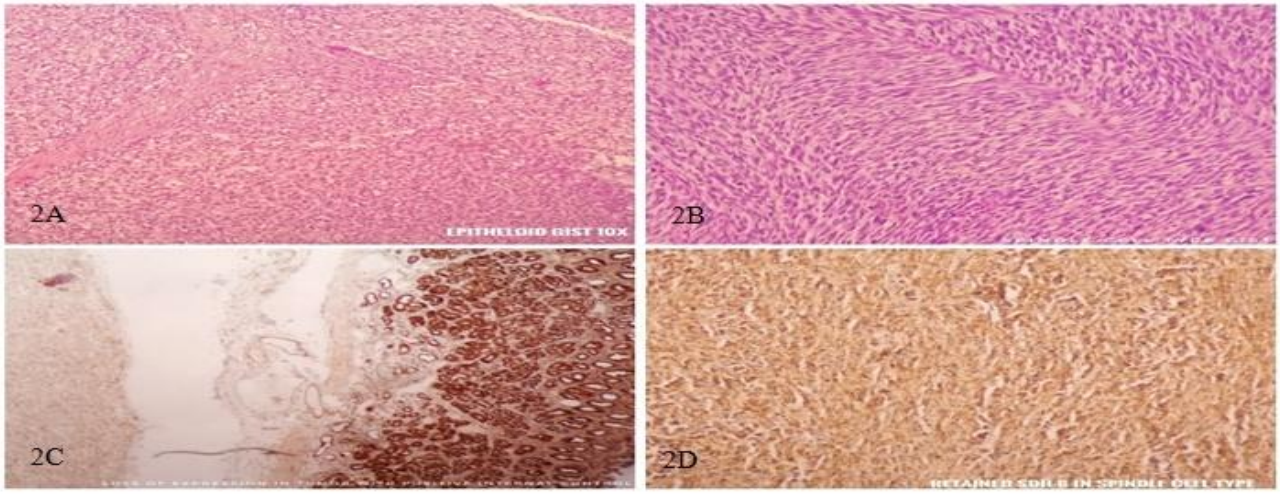

Figure-2: A: 10X Epithelioid variant of GIST, showing sheets of epithelioid cells with a condensed cytoplasmic rim adjacent to nucleus. Cells have well defined cell membranes, round nuclei and small nucleoli. B: 10 X, Spindle cell variant of GIST. Cells are arranged in short interlacing fascicles. Cells have pale to eosinophilic cytoplasm, minimal nuclear pleomorphism and cytoplasmic vacuoles which indent the nucleus. C: SDHB staining shows loss of expression in tumor cells. In contrast, gastric epithelium shows intact expression (positive internal control). D: Intact expression of SDHB in a case of spindle cell GIST.

About n=15 (23.8%) cases were gastric in origin in our study. A fifth of these cases were negative for SDHB. This number is far higher than a previous study by Miettinen *et al*, who

found SDHB mutations in only 7.5% of all the gastric GISTS he examined. However, it was a larger study with more than 700 cases of gastric GISTS[6]. Although, the current study might still imply that SDHB deficient GISTS are more prevalent in our society, a larger study is recommended to confirm this finding[5].

All of the cases in our study were under the age of 30 years which is younger than the average age of non-SDH deficient cases in our study. The median age of SDHB deficient tumors in the current study was 20 years. A similar trend was also reported by Miettinen *et al*, who reported a median age of 21 years in their study[5]. Most studies reported a female predominance in their study, but in our study there was male predominance as a whole.

All of SDH deficient patients showed epithelioid morphology in our study. In contrast, only 7.9% of non SDH deficient GISTS showed pure epithelioid features. The predominance of epithelioid phenotype is also apparent in most of the larger studies. No significant difference was found between tumor size, mitosis and risk category of SDH deficient and intact tumors. Most SDH deficient tumors are known to show prognosis independent of all these factors. CD117 and DOG1 were positive in all SDHB deficient GISTS. The Pediatric type GISTs (SDH-B negative) have been reported to have a more indolent course than adult GIST, and most studies have reported a female predominance. The tumor is frequently multi-focal and shows frequent recurrence. There is an increased incidence of nodal and visceral metastasis, including liver and abdominal sites. The tumor responds poorly to the conventional therapies and therefore identification of this tumor is of paramount importance[8-9].

This is the first study on the expression of SDHB in cases of GISTs in Pakistan and we hope that expression of SDHB will become an integral part of diagnostic and treatment protocol for management of GIST.

CONCLUSION

Immunohistochemistry is a reliable and cost-effective method for detecting mutation in SDH gene. Based on findings of this study it is recommended that all gastric GISTs below the age of 30 years with epithelioid morphology should be tested for SDH deficiency.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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