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**Immunohistochemical Detection of Cluster of Differentiation 117(CD117)Tumor Marker in Gastrointestinal Stromal Tumors**

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**ABSTRACT**

This is a descriptive retrospective study aimed to evaluate the expression of cluster of differentiation molecule 117 (CD117) in patients with gastrointestinal stromal tumors using immunohistochemical method. Thirty formalin fixed paraffin block were collected from patients samples previously diagnosed as gastrointestinal stromal tumors at Radiation and Isotope Center-Khartoum- Sudan (7 of them were malignant gastrointestinal stromal tumors and 23 them were benign gastrointestinal stromal tumors). The formalin fixed paraffin blocks were cut by rotary microtome, and then stained by immunohistochemical method for detection of CD117. The data obtained were analyzed using SPSS program. The age of study samples ranged between one to 75 years with mean age of 51 years. The study revealed that the most patients were older than 50 years representing 19 (63.3%) and the remaining 11(36.7%) were younger than 50 years. Out of thirty samples the study showed that the male female ratio is 3:1. CD117 result were positive in 28 (93.3%), and negative in 2 (6.7%). Concerning the association between CD117 and biological behaviour of tumor in study samples revealed that 22 (73.3%) positive expression and 1 (3.3%) were benign, 6 (20%) positive expression and 1(3.3%) negative expression were malignant. The study concluded that the CD117 expression showed positive result in gastrointestinal stromal tumors, with no statistical association with the biological behaviour of the tumor, benign or malignant.

**المستخلص**

هدفت هذه الدراسة الوصفية التراجعية إلى تقييم افراز واسمة الأورام اللحمية المعوية (CD117) لدي المرضى المصابين بالاورام اللحمية المعوية باستخدام كيمياء الأنسجة المناعية. تم جمع ثلاثين قالب مثبت بالفورمالين من عينات مرضي مشخصين مسبقا علي انهم مصابين بالاورام اللحمية المعوية (7 منهم كانوا مصابين بالاورام اللحمية المعوية الخبيثة و 23 منهم بالاورام اللحمية المعوية الحميدة). تم قطع المقاطع من القوالب بالمشراح الدوار وصبغها بطريقة كيمياء الانسجة المناعية للكشف عن واسمة الاورام اللحمية المعوية (CD117). تراوحت أعمار المرضى بين عام واحد الي 75 عام بمتوسط عمر بلغ 51 سنة. أظهرت الدراسة أن معظم المصابين كانت أعمارهم أكثر من 50 سنة وكان عددهم 19 مريضاً (63.3%) و 11 مصاباً (36.7%) كانت أعمارهم اقل من 50 سنة. من أصل ثلاثين عينة أظهرت الدراسة أن نسبة اغلب الذكور للاناث كانت 3:2. أظهرت الدراسة أن CD117 كان ايجابي في 28 (93.3%) وسلب في 2 (6.7%).

اماالعلاقة بين افراز CD117 وسلوك الورم كان كالاتي:22 (73.3%) عينة موجبة وعينة واحدة (3.3%) سالبة كانت اورام حميدة و 6 (20%) موجبة وعينة واحدة (3.3%) سالبة كانت اورام خبيثة. خلصت الدراسة الى أن افرازCD117يعطي نتيجة ايجابية مع اورام اللحمية المعوية مع عدم وجود علاقة مع سلوكالورم (حميد او خبيث).

**KEYWORDS:** *Benign GISTs, malignant GISTs, CD 117*

## INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are subset gastrointestinal mesenchymal tumors of varying differentiation. There are approximately 3500-5000 cases of GIST per year in the United States. This makes GIST the most common form of stromal tumors, in all forms constitutes less than 1% of all cancer <sup>(1)</sup>. Previous reports from Sudan showed that these tumors were uncommon. Most of the cases were reported before endoscopy was introduced in the country in the last twenty years<sup>(2)</sup>. The risk factors and etiology of GISTs remain undetermined <sup>(3)</sup>, but more than 75% of the patients older than 50 years of age <sup>(1)</sup>. Computed tomography (CT) (in particular spiral CT scan with thin cuts) is the most sensitive test for the detection and staging of a primary gastrointestinal sarcoma, in respect to other imaging tests (upper gastrointestinal contrast study, abdominal ultrasound scanning, angiography)<sup>(4)</sup>. Specific immunohistochemical staining is used to support the diagnosis. While surgery is the main course of treatment for resectable GISTs <sup>(5)</sup>. Imatinib mesylate (Gleevec), a receptor tyrosine kinase (RTK) inhibitor, which was originally developed for the treatment of chronic myeloid leukaemia, has proved to be an effective treatment of GIST <sup>(6)(7)</sup>. Its action directly suppresses c-kit expression. A CD117 immunochemical positive tumor therefore indicates imatinib sensitivity <sup>(6)(8)</sup>. CD117/ c-kit; a trans-membrane tyrosine kinase growth factor receptor involved in cellular differentiation; is expressed

by the interstitial cells of cajal (ICC), melanocytes, mast cells and in GISTs <sup>(7)(9)</sup>. Mutations in the receptor tyrosine kinase (RTK) genes c-kit causes activation of tyrosine kinase, which promote proliferation and decrease apoptosis. Therefore, the CD117 expression by GIST plays a key role in the diagnosis and treatment of the patients <sup>(10)(11)</sup>.

## MATERIALS and METHODS

### Slides preparation

One section of 5µm thickness was obtained from each formalin fixed paraffin embedded tissue from Radiation and Isotope Center-Khartoum- Sudanusing a rotary microtome for immunohistochemistry which is then taken in thermal coated slides and dried in hot plate oven at 80°C for one hour <sup>(12)</sup>.

### Immunohistochemical stain

Sections were brought to water and retrieved using water bath retrieval technique at 97°C, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffer saline (PH 7.4) for five minutes, then treated with anti CD117 primary antibody for thirty minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for thirty minutes, then rinsed in phosphate buffer saline, then treated with DAB for seven minutes, then washed in phosphate buffer saline for five minutes, then counterstained in Mayer's haematoxylin for one minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending of

ethanol (50%, 70%, 90%, 100%) two minutes for each then cleared in two change of xylene two minutes for each, and mounted in DPX mounting media (12).

**RESULTS**

In this study patient's ages was classified into three groups; 11 (36.7%) of them were less than 50 years, 16 (53.3%) were between 50 and 70 years and 3 (10%) were more than 70 years (Table 1).

The sex of subject revealed that 18 (60%) of them were males and the remaining 12 (40%) were females (Table 2). The immunohistochemical expression of CD117 among study samples showed 22 (73.3%) positive expression and 1 (3.3%) were benign, 6

(20%) positive expression and 1(3.3%) were malignant (Table 3).

Figure (1) shows a section from epigastric malignant gastrointestinal stromal tumor mass with positive expression of CD117 (40x).

Figure (2) shows a section from abdominal benign gastrointestinal stromal tumor mass with positive expression of CD117 (40x)

Also figure (3) shows asection from intestinal benign gastrointestinal stromal tumor biopsy with negative expression of CD117 (40x) while figure (4) shows asection from gastric malignant gastrointestinal stromal tumor mass have negative expression of CD117 (40x)

*Table(1): Distribution of age groups among the study samples*

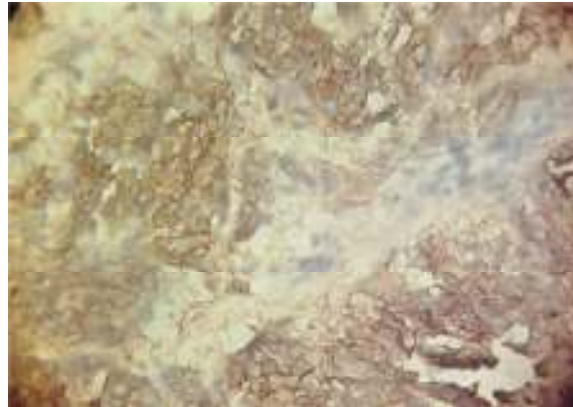
| Age group (year) | Frequency | Percent |
|------------------|-----------|---------|
| < 50             | 11        | 36.7    |
| 50-70            | 16        | 53.3    |
| > 70             | 3         | 10.0    |
| Total            | 30        | 100.0   |

*Table(2): Frequency distribution of sex among study samples*

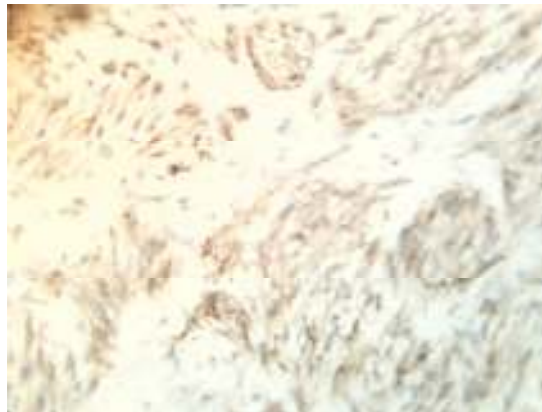
| Sex    | Frequency | Percent |
|--------|-----------|---------|
| Male   | 18        | 60.0    |
| Female | 12        | 40.0    |
| Total  | 30        | 100.0   |

*Table (3): Relation between CD117 expression and biological behavior of the tumor*

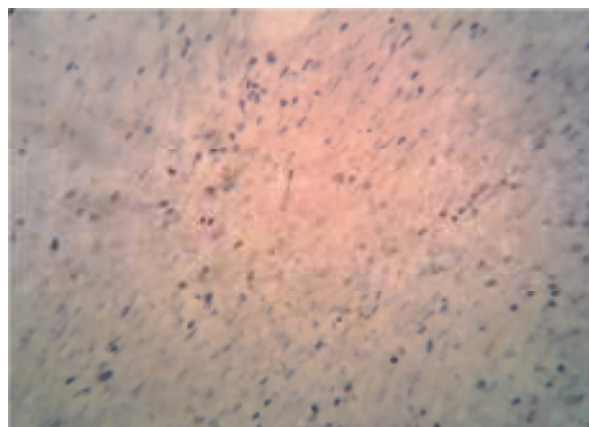
| Tumor behavior | CD117     |          | Total     | P. value |
|----------------|-----------|----------|-----------|----------|
|                | Positive  | Negative |           |          |
|                | N (%)     | N (%)    | N (%)     |          |
| Benign         | 22 (73.3) | 1 (3.3)  | 23 (76.7) | 0.356    |
| Malignant      | 6 (20)    | 1 (3.3)  | 7 (23.3)  |          |
| Total          | 28 (93.3) | 2 (6.7)  | 30 (100)  |          |



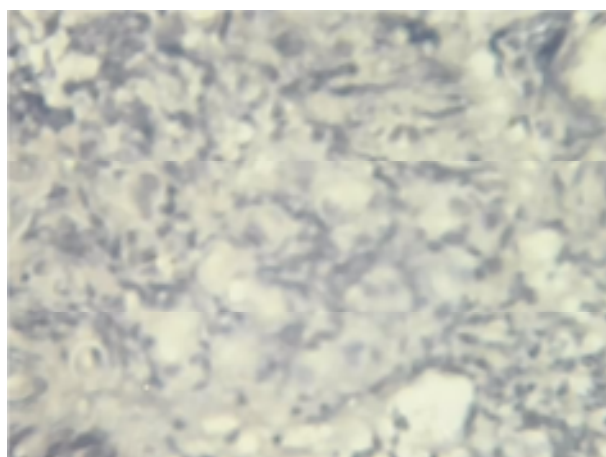
**Figure (1):** Section from epigastric malignant gastrointestinal stromal tumor mass shows positive expression of CD117 (40x)



**Figure (2):** Section from abdominal benign gastrointestinal stromal tumor mass shows positive expression of CD117 (40x)



**Figure (3):** Section from intestinal benign gastrointestinal stromal tumor biopsy shows negative expression of CD117 (40x)



**Figure (4):** Section from gastric malignant gastrointestinal stromal tumor mass shows negative expression of CD117 (40x)

## DISCUSSION

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumor of the gastrointestinal tract<sup>(13)</sup>.

In this study out of thirty patients diagnosed with gastrointestinal stromal tumors from Radiation and Isotope Center-Khartoum –Sudan were investigated by histopathology and immunohistochemistry.

Histopathological analysis of tumor behavior in the 30 cases of gastrointestinal stromal tumors revealed high percent in benign tumor 23 (76.7%) and 7 (23.3%) were malignant. This result supported by Nishida and Hirota,<sup>(14)</sup> they reported that about 10-30% of GISTs have malignant behaviour. Also Miettinen and Lasota<sup>(15)</sup> have estimated that up to 30% of all GISTs are malignant.

Distribution of age among patients has been evaluated; as the mean age was 51 years and it was noted that patients diagnosed with gastrointestinal stromal tumors with ages older than 50 years were 19 (63.3%) compared to their counterparts with ages younger than 50 (36.7%) indicating that incidence with gastrointestinal stromal tumors increased with age; this result agree with Dematteo, *et al.*<sup>(16)</sup> who reported

that tumor occur at any age but 75% are diagnosed in >50 year-olds. This result not far away to the finding of Nowain, *et al.*<sup>(17)</sup> who reported that most patients present between the ages of 50 and 80 years. Also Miettinen, *et al.*<sup>(18)</sup> reported that GIST is affected with a wide age distribution, although more than 75% of the patients older than 50 years of age. Based on this study gastrointestinal stromal tumors were predominant in males 18 (60%) and 12 (40%) in females. These agree with Miettinen and Lasota<sup>(15)</sup> who reported that some series show a male predominance, and others show equal gender distribution. Also Trans, *et al.*<sup>(19)</sup> reported that there is predominance of GISTs among men. Similar result of Nilsson, *et al.*<sup>(20)</sup> who reported that GIST tumors slightly more predominant in men than women. Also kim, *et al.*<sup>(21)</sup> concluded that the incidence between the sexes is the same, although a study reported that there is a slight predominance in men. This result disagree with Nowain, *et al.*<sup>(17)</sup> (2005) who reported that GISTs are equally distributed across all geographic and ethnic groups and men and women are equally affected.

The analysis of immunohistochemical expression of CD117 in 30 cases of this

study revealed that most staining results were positive expression representing 28 (93.3%) and the remaining 2 (6.7%) were negative CD117 expression. This result supported by Fletcher,*et al.* <sup>(22)</sup> who reported that the proportion of CD117 positive cells in GISTs is usually at least 90%. Similar result of Medeiros, *et al.* <sup>(23)</sup> who reported that CD117 immunostains positively in GISTs with very rare exceptions, due to artifacts, sampling errors, or lack of c-KIT caused by clonal evolution or mutations. Also Miettinen and Lasota, <sup>(1)</sup> reported that there remains a small problematic group of tumors with morphological features of GISTs without expression of CD117.

Based on this study the statistical association between CD117 expression and tumor behaviour is insignificant (P value > 0.05), this result supported by Sarlomo-Rikala, *et al.* <sup>(24)</sup> whom study reported that CD117 was almost always (85%) expressed in both benign and malignant GISTs. Also Miettinen, *et al.* <sup>(25)</sup> reported that CD117 positivity is seen in all histologic variants and in benign and malignant GIST of different sites. Also Fletcher, *et al.* <sup>(22)</sup> reported that expression of CD117 is seen in almost all GISTs regardless of the site of origin, histologic appearance and biologic behaviour, and is therefore considered to be best defining feature of GISTs. Similar findings reported by Hornick and Fletcher, <sup>(26)</sup>.

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