Immunohistochemichal Expression of DOG1 as a Diagnostic Marker for Gastrointestinal Stromal Tumors in Comparison to c-KIT

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Abstract: Background: Accurate diagnosis of gastrointestinal stromal tumor (GIST) has become imperative because of availability of treatment by tyrosine kinase inhibitors (TKIs). About 4% to 15% of GISTs show weak or negative staining for c-KIT/CD117. Diagnosis of these tumors remains a significant challenge. Studies have suggested that antibodies against DOG1 could serve as specific immunohistochemical markers for GIST irrespective of the underlying mutation or c-KIT expression by IHC. **Aim of the work:** To evaluate the diagnostic efficacy of DOG1 in comparison to c-KIT in GIST. **Material and Methods**: Immunohistochemical (IHC) staining for 51 GISTs was performed using c-KIT and DOG1 antibodies. Other mesenchymal tumors (13 cases) were included in the study and stained for both markers to test for their sensitivity, specificity and diagnostic accuracy. **Results**: Out of the 51 cases of GISTs, 35/51 (68.6%) cases were positive for both c-KIT and DOG1 antibodies. Thirteen cases were DOG1-positive c-KIT-negative. Three cases were DOG1-negative c-KIT-negative. A statistically significant concordance was found between c-KIT and DOG1 immunoreactivity (p=0.008), with mild agreement between the two markers (kappa=0.24). For c-KIT, the PPV for the diagnosis of GIST was 100%, the NPV was 44.8%, the overall diagnostic accuracy was 75%, with a sensitivity and specificity of 68.6 % and 100% respectively. As for DOG1, the PPV was 100%, the NPV was 81.3%, the overall diagnostic accuracy was 95.3% with a sensitivity and specificity of 94.1% and 100% respectively.
Conclusions: DOG1 is a more sensitive immunohistochemical marker for GIST than c-KIT and we recommend using DOG1 as the first choice antibody for the diagnosis of GIST.
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1. Introduction:

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal neoplasm of the gastrointestinal tract **(Laurini and Carter, 2010)**. Approximately 85% of GISTs harbor activating mutations in the c-KIT receptor tyrosine kinase (RTK) gene or the homologous RTK platelet-derived growth factor receptor alpha (PDGFRA) gene **(Hirota *et al.*, 2001; Heinrich *et al.*, 2003).** Inhibition of c-KIT and PDGFRA by tyrosine kinase inhibitors has revolutionized the treatment of GISTs and demands accurate tumor classification **(Liegl *et al.*, 2009).**

Mutation screening of c-KIT or PDGFRA can serve in confirming the diagnosis of GIST, but only a few centers worldwide perform this analysis clinically **(Debiec-Rychter *et al.*, 2004).** CD34 is not a specific marker for GIST and is positive in many other soft tissue tumors that may enter into the differential diagnosis of GIST. Consequently, its utility in the diagnosis of GIST is limited (**Espinosa *et al.*, 2008**). In contrast, c-KIT is a relatively specific IHC marker for GIST. However, about 4% to 15% of GISTs show weak or negative staining for c-KIT/CD117 and hence may remain undiagnosed as GIST **(Medeiros *et al.*, 2004)**. Many of these "c-KIT-negative" GIST possess PDGFRA mutations and a subset of these cases is sensitive to treatment with imatinib. Diagnosis of these tumors remains a significant challenge. **(Corless *et al.*, 2005; Espinosa *et al.*, 2008**). It is further complicated by the controversy in the literature about definition of c-KIT negativity by IHC.

Discovered on GIST-1 (DOG1) genes were identified as a typical finding in gene expression profiling studies on GISTs **(West *et al.*, 2004).** The corresponding DOG1 protein has been identified as a calcium regulated chloride channel protein (**Caputo *et al.*, 2008; Yang *et al.*, 2008**). Studies have suggested that antibodies against DOG1 have superior sensitivity and specificity compared with c-KIT, and that these antibodies could serve as specific IHC markers for GIST irrespective of the underlying mutation or c-KIT expression by IHC. DOG-1 staining would be an essential tool for a more reliable diagnosis on GISTs especially c-KIT-weak/negative tumors (**West *et al.*, 2004; Espinosa *et al.*, 2008; Liegl *et al.*, 2009).**

The aim of this study was to evaluate immunohistochemical expression of DOG1 as a diagnostic marker for GIST. We also compared

immunohistochemical staining and diagnostic efficacy of DOG1with that of c-KIT in GIST.

2. Material and methods

Tumor samples:

This retrospective study was conducted on 51 GIST biopsies of Egyptian patients obtained during the period between 2005 to 2011 from Pathology Department, Faculty of Medicine, Menoufiya University, Egypt. The clinical and follow-up data (gender, age, medical history, site of tumor, recurrence) were obtained from the medical records.

The hematoxylin and eosin stained sections were prepared from the formalin-fixed paraffin embedded tissue blocks and reviewed to confirm the diagnoses before inclusion in the study. We evaluated: tumor size, tumor cell types (spindle, epithelioid, or mixed), tumor cellularity (hypo, moderate or hypercellularl), tumor vascularity (low, moderate or high), mitotic rate [expressed as the number of mitotic figures/ 50 high-power fields (HPFs) in the most mitotic area, using a 40 objective and a 10 ocular; field size 0.25mm2], Necrosis (present or absent) and lymph node involvement (positive or negative). Risk stratification was performed considering anatomic site in addition to size and mitotic activity according to the 2007 National Comprehensive Cancer Network guidelines **(Demetri *et al.*, 2007)**

Immunohistochemical (IHC) staining: The IHC staining was performed on the 4 μm thick sections of formalin-fixed, paraffin-embedded blocks. The standard streptavidin-biotin amplified system was used. The antibodies, clones, dilutions, pretreatment conditions, and sources are listed in table (1). We used the Envision Plus detection system (Dako, Carpinteria, CA) for all antibodies. Appropriate positive and negative controls were included. The results on DOG1 immunostaining were tabulated blindly without knowing the results on c-KIT. We graded immunoreactivity of both DOG1 and c-KIT semiquantitatively as 0: no staining; 1+: <5% tumor cells reactive; 2+: 5%to 25% of tumor cells reactive; 3+: >25% to 50% tumor cells reactive; and 4+: >50% tumor cells reactive **(Liegl *et al.*, 2009).** For statistical purpose (in comparing expression of DOG1 with clinicopathologic parameters): scores 0, 1 and 2 were lumped together (as low scores) and scores 3 and 4 were lumped together (as high scores).

Table (1): Panel of antibodies used in this study

Antigen	Clone	Dilution	Antigen retrieval	Source
c-KIT	Polyclonal	Ready to use	HIER, Citrate pH 6	Genemed Biotechnologies
DOG1	Monoclonal	1:100	HIER, EDTA pH 8	Thermo Scientific
CD34	Monoclonal	Ready to use	HIER, Tris pH 9	Genemed Biotechnologies
SMA	Monoclonal	Ready to use	HIER, EDTA pH 8	Thermo Scientific
Desmin	Monoclonal	Ready to use	HIER, EDTA pH 8	Thermo Scientific
S100	Monoclonal	Ready to use	None	Thermo Scientific

DOG1: Detected on Gastrointestinal Stromal Tumor; SMA: Smooth Muscle Actin; HIER: Heat Induced Epitope Retrieval

Mesenchymal tumors other than GIST were included in the study and stained with both c-KIT and DOG1.1 to test for their specificity. Hence, 11 leiomyomas, 1 dermatofibroma and 1 dermaofibrosarcoma protuberance were retrieved from the archives and stained with both markers.

The numbers of true-positive (TP), true negative (TN), false-positive (FP), and false-negative (FN) results were determined for each of the two tested markers. By determination of the earlier parameters, the sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and diagnostic accuracy of each marker were determined.

Statistical analysis:

Data were collected, tabulated and statistically analyzed using a personal computer with "statistical package for the social sciences" version 16 program. Chi-square and Fisher's exact tests were used in comparison between qualitative variables. Mann–Whitney (U) and Student t (t) tests were used in comparison between quantitative variables. $P \leq 0.05$ was considered significant.

3. Results:

Clinicopathologic Features:

The clinical and histopathological data of the studied cases are illustrated in table (2). All c-KIT-negative GISTs included in this study showed classic morphologic features, and fulfilled previously described parameters **(Liegl *et al.*, 2009).** Proper risk assessment was not possible in 10/51 cases because of limited clinical data (regarding tumor size or its anatomic site).

c-KIT immunostaining:

Out of the 51 cases of GIST included in this study, 6 cases (11.8%) were completely negative (score 0) for c-KIT. Ten cases (19.6%) had a score of 1+ (<5% positive staining), 10 cases had a score of 2+ (19.6), 10 cases had a score of 3+ (19.6%) and the remaining 15 cases(29.4%) had a score of 4+. All the positive cases showed cytoplasmic expression with occasional additional membranous staining.

When we consider 1+ group (<5% positive staining) as positive, no statistically significant concordance was found between c-KIT and DOG1 immunostaining scores (*P*=0.56) with slight agreement (Kappa=0.08) (data are not shown). On the other hand on considering 1+ group as negative, a statistically significant concordance was found between c-KIT and DOG1 immunostaining scores (*P*=0.008) with fair agreement (Kappa=0.24) (Table 3). Furthermore, 3/13 cases of the control group (3 leiomyomas) showed scattered positive c-KIT expression in <5% of the tumor cells (score 1+) (figure) while the remaining 9/13 cases were completely c-KIT negative (score 0). So we add 1+ group to 0 group and consider both as negative c-KIT in cases [16/51 (31.4%)] and in control [13/13 (100%)] while 2+, 3+, and 4+ groups were considered as positive c-KIT [35/51 (68.6%) GIST cases].

DOG1 immunostaining:

Out of the 51 cases of GIST included in this study, 48 cases (94.1%) were positive for DOG1(six cases had a score of 2+, 4 cases had a score of 3+ and the remaining 38 cases had a score of 4+). The remaining 3 cases (95.9%) were completely DOG1 negative (score 0). One of the 3 negative cases was located in the small intestine and the other 2 were located in the retroperitonium. The pattern of staining of DOG1was membranocytoplasmic (Fig. 3B) in 26 cases and cytoplasmic only in 22 cases(Fig. 3B). The intensity of expression was mild in 14 cases, moderate in 22 cases and strong in 12 cases. All the 13 control cases (100%) were negative for DOG1 (score 0).

DOG1 IHC Profile in c-KIT-positive GISTs:

All the 35 (100%) c-KIT-positive tumors also expressed DOG1(30 tumors with 4+, 3 with 3+, one with 2+, and one with 1+ staining). Additional immunohistochemical evaluation revealed that CD34 was expressed in 11/19 (57.9%) cases, smooth muscle actin (SMA) in 15/19 (78.9%), desmin in 0/11 (0%), and S-100 protein expression was seen in 1/18 cases (5.6%).

DOG1 IHC Profile in c-KIT-negative GISTs:

In the group of GISTs lacking c-KIT expression, 13/16 cases (81.25%) were DOG1-positive (8 tumors with 4+, one with 3+, and 4 with 1+ staining). Only three cases (18.75%) of c-KIT-negative tumors were also DOG1-negative. Additional immunohistochemical evaluation revealed that CD34 was expressed in 10/13 (76.9%) cases, smooth muscle actin (SMA) in 7/11 (63.6%), desmin in 1/6 (16.7%), and S-100 protein expression was seen in 1/5 cases (20%). In the c-KIT and DOG1-negative cases at least focal CD34 expression in association with SMA and/or desmin was detected.

Table(2): Clinicopathologoical features of the studied cases

	The studied cases N = 51 N (%)
Age	
X ± SD	54.73±14.04
Median (Range)	57 (23 – 79)
Age group	
< 40	8 (15.7)
≥ 40	43(84.3)
Sex	
Male	21 (41.2)
Female	30 (58.8)
Size	
X ± SD	17.0±6.70
Median (Range)	15 (4 – 35)
Size	N=41
≤ 10cm	4 (9.8)
> 10cm	37 (90.2)
Missing	10
Site	
Stomach	13 (22.5)
Large intestine	7 (13.7)
Small intestine	6 (11.8)
Others	25 (49.0)
Morphology	
Spindle	41 (80.4)
Epithelioid	2 (3.9)
Mixed	8 (15.7)
Cellular	
Hypo	14 (27.5)
Moderate	13 (25.5)
Hyper	24 (47.1)
Vascularity	
Low & moderate	29 (56.9)
High	22 (43.1)
Mitosis	
> 5/50	28 (54.9)
≤5/50	23 (45.1)
Risk Stratification	N=41
Non high risk	6 (14.6)
High risk	35 (85.4)
Missing	10
Necrosis	
Present	33 (64.7)
Absent	18 (35.3)
LN	N=9
Positive	3 (33.3)
Negative	6 (66.7)
Missing	15
Recurrence	
Recurrent	7 (13.7)
Not	44 (86.3)

N: Number

Diagnostic efficacy of c-KIT & DOG1 in GISTs:

As regards c-KIT immunostaining, the PPV for the diagnosis of GIST was 100%. The NPV was 44.8%. The overall diagnostic accuracy was determined to be 75%, with a sensitivity and

specificity of 68.6 % and 100% respectively. As for DOG1.1, the PPV for the diagnosis of GIST was 100%. The NPV was 81.3%. The overall diagnostic accuracy was determined to be 95.3%, with a sensitivity and specificity of 94.1% and 100% respectively. Table (4).

Correlation between c-KIT & DOG1 expression and the studied clinicopathologic parameters:

When we compare positive and negative c-KIT together with low and high scores of DOG1against the clinicopathologic parameters of GIST, only the patients sex achieved a significant correlation with c-KIT expression (P=0.0005). The majority of male patients (90.5%) showed high scores of c-KIT. Table (5).

Table (3): Relation between KIT and DOG1 immunostaining.

	DOG1			Kappa	*P* value
	Positive N = 48 No (%)	**Negative N = 3 No (%)**	**Total N = 51 No (%)**		
c-KIT					
Positive	35(72.9)	0(0)	35(68.6)	0.24	0.008*
Negative or focal	13(27.1)	3(100)	16(31.4)		

*: Significant

Table (4): Diagnostic Efficacy of both c-KIT and DOG1.1 in GISTs.

	Groups			Groups	
	Case N = 51 N (%)	**Control** N = 13 N (%)		**Case** N = 51 N (%)	**Control** N = 13 N (%)
c-KIT			**DOG1**		
Positive	35(68.6)	0(0)	Positive	48(94.1)	0(0)
Negative	16(31.4)	13(100)	Negative	3(5.9)	13(100)
Sensitivity	68.6		**Sensitivity**	94.1%	
Specificity	100		**Specificity**	100%	
PPV	100		**PPV**	100%	
NPV	44.8		**NPV**	81.3	
Accuracy	75.0		**Accuracy**	95.3%	

PPV: Positive predictive value; NPV: Negative predictive value; N: Number

Table (5): Correlation between DOG1 and c-KIT expression and the studied clinicopathologic parameters

Variables	DOG1 expression		Test of sign. & P value	c-KIT		Test of sign.& P value
	High scores N = 42 N (%)	**Low scores N = 9 N (%)**		**Positive N = 35 N (%)**	**Negative N = 16 N (%)**	
Age						t-test=0.68
X ± SD	52.44±14.53	52.44±14.53	U=0.51	55.63±13.55	52.75±15.26	P=0.50
Median(Range)	51(33 – 79)	51(33 – 79)	P=0.61	57(23 – 78)	54.5(29 – 79)	
Age group						
< 40	6(75.0)	2(25.0)	FE=0.35	4(50)	4(50)	FE=1.53
≥ 40	36(83.7)	7(16.3)	P=0.62	31(72.1)	12(27.9)	0.24
Sex						
Male	18(85.7)	3(14.3)	FE=0.28	19(90.5)	2(9.5)	X2 =7.92
Female	24(80.0)	6(20.0)	P=0.72	16(53.3)	14(46.7)	P=0.005*
Size N=41						
X ± SD	18.78±6.94	18.78±6.94	U=0.10	16.21±6.76	18.69±6.5	U=1.2
Median(Range)	16(12 – 35)	16(12 – 35)	P=0.32	15(4 – 30)	16(12 – 35)	P=0.23
Size N=41						
≤ 10cm	4(100)	0(0)	FE=1.25	4(100)	0(0)	FE=2.06
> 10cm	28(75.7)	9(24.3)	P=0.56	24(64.9)	16(35.1)	P=0.29
Site						
Stomach	12(92.3)	1(7.7)	X2=2.05	8(80)	2(20)	X2=1.31
Large intestine	6(85.7)	1(14.3)	P=0.56	3(100)	0(0)	P=0.73
Small intestine	4(66.7)	2(33.3)		3(100)	0(0)	
Others	20(80.0)	5(20.0)		11(84.6)	2(15.6)	
Morphology						
Spindle	35(85.4)	6(14.6)	FE=1.31	27(65.9)	14(34.1)	FE=0.75
Epithelioid & Mixed	7(70)	3(30.0)	P=0.35	8(80)	2(20)	P=0.47
Cellular					4(28.6)	
Hypo	11(78.6)	3(21.4)	X2=3.11	10(71.4)	6(46.2)	X2=1.82
Moderate	9(69.2)	4(30.8)	P=0.21	7(53.8)	6(25)	P=0.4
Hyper	22(91.7)	2(8.3)		18(75)		

Vascularity						
Low- Moderate	23(79.3)	6(20.7)	FE=0.43	17(58.6)	12(41.4)	FE=3.13
High	19(86.4)	3(13.6)	P=0.71	18(81.8)	4(18.2)	P=0.08
Mitosis						
> 5/50	24(85.7)	4(14.3)	FE=0.48	22(78.6)	6(21.4)	FE=2.85
≤5/50	18(78.3)	5(21.7)	P=0.71	13(56.5)	10(43.5)	P=0.09
Risk stratification						
Non- high risk	5(83.3)	1(16.7)	FE=0.12	4(66.7)	2(33.3)	FE=0.009
High risk	27(77.1)	8(22.9)	P=1	24(68.6)	11(31.4)	P=1
Necrosis						
Present	28(84.8)	5(15.2)	FE=0.40	24(72.7)	9(27.3)	FE=0.73
Absent	14(77.8)	4(22.2)	P=0.70	11(61.1)	7(38.9)	P=0.39
LN						
Positive	1(33.3)	2(66.7)	FE=0.90	1(33.3)	2(66.7)	FE=0.23
Negative	4(66.7)	2(33.3)	P=0.52	3(50)	3(50)	P=1
Recurrence						
Recurrent	6(85.7)	1(14.3)	FE=0.06	6(85.7)	1(14.3)	FE=1.57
Not	36(81.8)	8(18.2)	P=1	29(65.9)	15(34.1)	P=0.40

X ± SD: mean ± standard deviation; N: Number; t- test: Student t test; *: Significant; N: Number; **U**=Mann-Whitney test χ^2: Chi-square test; LN: Lymph node; FE: Fisher's Exact

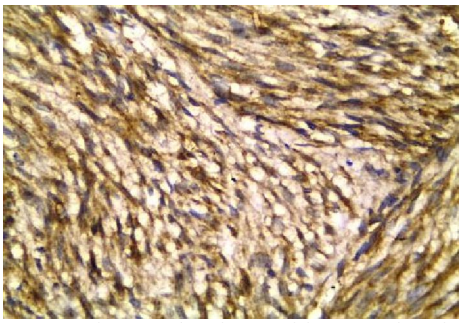


Fig.1: A case of GIST showing positive cytoplasmic c-KIT expression (immunoperoxidase method x400).

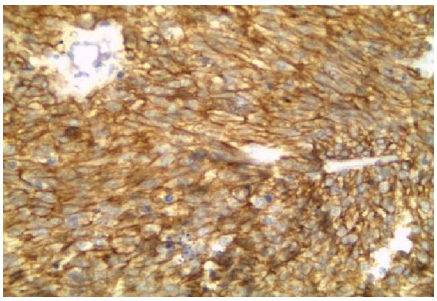


Fig.2: A case of GIST showing positive membranocytoplasmic DOG1 expression (immunoperoxidase method x400).

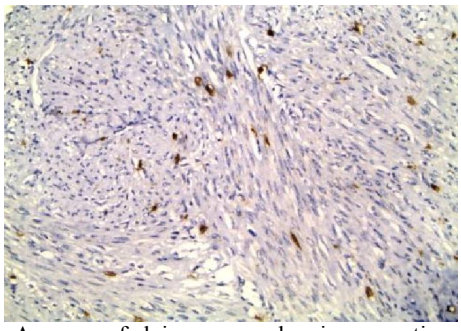


Fig.3: A case of leiomyoma showing negative c-KIT expression (few scattered positive cells <5% of the tumor) (immunoperoxidase method x200).

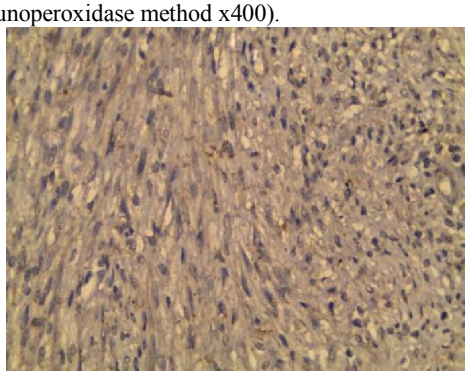
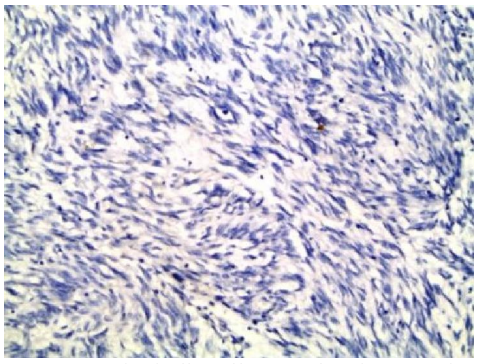


Fig.4: A case of leiomyoma showing negative DOG1 expression (immunoperoxidase method x400).



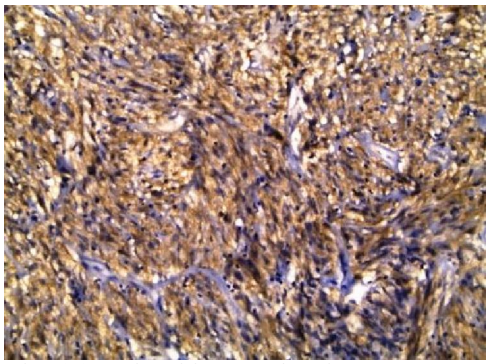


Fig.5 A&B: A case of spindle cell GIST showing negative c-KIT expression (A), and positive cytoplasmic DOG1 expression (B) (immunoperoxidase method x200).

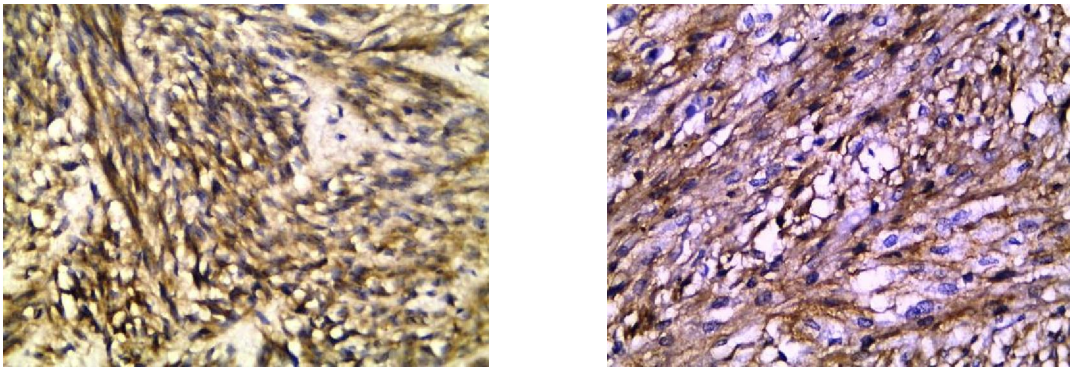


Fig.6 A & B: Another case of Spindle cell GIST showing positive cytoplasmic c-KIT expression (A), and positive cytoplasmic DOG1 expression (B) (immunoperoxidase method x400).

4. Discussion:

With the recent development of effective targeted therapies for GIST, the correct diagnosis of these tumors has a considerable clinical impact and great importance. The use of TKIs has led to a dramatic improvement in the survival rates of GIST patients, in addition to improving their quality of life **(Kang *et al.*, 2010)**. Most GIST can be identified based on the combination of tumor location, histologic appearance, and the presence of c-KIT by immunohistochemistry. In the vast majority of GISTs, high levels of c-KIT expression are accompanied by a c-KIT gene mutation **(Rubin *et al.*, 2001; Hirota *et al.*, 2001).** A subset of GISTs have been found to have PDGFRA mutations rather than c-KIT mutations **(Hirota *et al.*, 2003; Heinrich *et al.*, 2003a).** These patients may still benefit from imatinib therapy, but their tumors often fail to react with antibodies against c-KIT and hence may remain undiagnosed as GIST **(Heinrich *et al.*, 2003b)**. In addition, some GISTs with c-KIT mutations may have low c-KIT expression by IHC yet will still respond to imatinib therapy **(Bauer *et al.*, 2003).** Screening for c-KIT and PDGFRA mutations can be helpful in this setting, but this approach adds to the time and cost of diagnosis and only a few centers worldwide perform this analysis clinically. What is needed to aid in routine diagnosis is a marker that reliably stains GIST that are c-KIT-weak/negative **(Espinosa *et al.*, 2008)**.

DOG1 is a calcium regulated chloride channel protein that was found to be selectively expressed in GIST independent of c-KIT/PDGFRA mutation status (**West *et al.*, 2004; Espinosa *et al.*, 2008; Liegl *et al.*, 2009).** The aim of this study was to evaluate IHC expression of DOG1 as a diagnostic marker for GIST. We also compared IHC staining and diagnostic efficacy of DOG1with that of c-KIT in GIST.

In the current work, 16/51 (31.4%) cases were negative for c-KIT. The definition of c-KIT negativity in GIST was to some extent controversial. This may explain the variable range of GIST tumor positive for c-KIT in the literature which range from 74% to 98.1% **(Liu *et al.*, 2006; Espinosa *et al.*, 2008; Abdel-Hadi *et al.*, 2009; Liegl *et al.*, 2009; Kang *et al.*, 2010).** Some studies define c-KIT positivity as any positive percentage of cells regardless intensity of expression. They considered even focal (or weak) but convincing staining for c-KIT as a positive result **(Abdel-Hadi *et al.*, 2009; Liegl *et al.*, 2009)**. In others, the expression was scored as positive if ＞5% of the tumor cells were reactive for c-KIT with any intensity **(Kang *et al.*, 2010).** C-KIT positivity in other studies depends mainly on staining intensity rather than percentage. They considered positivity if any moderate or strong complete membranous c-KIT staining is noticed whether focal or diffuse in tumor **(Epsinosa *et al.*, 2008).** Finally still other studies emphasize a diffuse, strong c-KIT immunoreactivity for the diagnosis of GIST **(Fletcher *et al.*, 2002).** In our experience, we considered c-KIT expression as negative when focal staining in less than 5% of tumor cells was observed. We recommend using this cut off for several reasons: First all the focal cases showed also weak cytoplasmic staining; second a statistically significant concordance was found between c-KIT and DOG1 immunostaining scores (P=0.008) with fair agreement (Kappa=0.24) only with considering focal cases as negative; and third the focal pattern of c-KIT expression appear as a non - specific staining in three cases of the control group (3 leiomyomas) so we could not depend upon focal staining pattern for differentiating GIST from other mesenchymal tumors.

In the current study, we evaluated DOG1 antibody as a diagnostic marker for GISTs. The results demonstrated that DOG1 is a specific and sensitive marker for GIST, as it stained 48/51 (94.1%) cases of GIST included in the study and didn't stain any of the other mesenchymal tumors tested. All c-KIT-positive GISTs (35 cases) demonstrated positive staining with DOG1.Our results are in agreement with that done by

Abdel-Hadi *et al.* (2009) as 45/47 (95.7%) of their GIST cases were DOG1 positive. **West *et al.* (2004) and Kang *et al.* (2010)** demonstrated 97.8% and 90.7% DOG1 positivity in GIST respectively. **Liegl *et al.* (2009)** found that 61/81 (75.3%) of GISTs are positive for DOG1and that all c-KIT-positive GISTs were DOG1 positive. They confirmed that DOG1 is a very sensitive marker for GIST. **Espinosa *et al.* (2008)** demonstrated the high sensitivity and specificity of DOG1for GIST and showed that DOG1 reactivity was seen in 370/425 GISTs cases (87%), whereas only 1 leiomyosarcoma, 1 synovial sarcoma, and 1 desmoplastic malignant melanoma out of 935 soft tissue sarcomas expressed DOG1.

In the present work, we compared immunohistochemical staining and diagnostic efficacy of DOG1with that of c-KIT in GISTs. DOG1 proved to be a more sensitive marker than c-KIT for the diagnosis of GISTs (94.1% versus 68.6% respectively). Furthermore, 13/16 cases (81.25%) of the c-KIT-negative GISTs in the current work were DOG1-positive. In the Study done by **Espinosa *et al.* (2008)** DOG1 antibody identified 63 GISTs more than c-KIT and in the study by **Liegl *et al.* (2009)** DOG1 was positive in 36% of c-KIT-negative tumors. Both studies demonstrated that DOG1 is a more sensitive marker for GIST than c-KIT. **Abdel-Hadi *et al.* (2009)** found that DOG1 identified only one case that was c-KIT-negative. Finally, although both markers were proved to be 100% specific for GIST in the present study, DOG1 have a diagnostic accuracy of 95.3% compared to 75% for c-KIT. This results may magnify the importance of DOG1in our work that may be able to pick up a large numbers of c-KIT-negative cases and diagnose them as GIST.

In the present study, a statistically significant concordance was found between the results of c-KIT and DOG1immunoreactivity (P=0.008) with mild agreement between the two markers (K= 0.24). Thirty five (68.6%) cases of GISTs were positive for both markers. Although this figure is higher than that reported by **Liegl *et al.* (2009)** (62.96%), it is much lower than that reported by **Miettinen *et al.* (2009)** (92.3%) and **Abdel-Hadi *et al.* (2009)** (93.6%). The great difference may be related, in part, to the different methods for assessing c-KIT positivity.

The biological behavior of GIST is difficult to predict because some GISTs metastasize, whereas others remain asymptomatic for years **(Kim *et al.*, 2004).** Although various clinicopathologic criteria for making the prognosis have been suggested, there is not enough information about the correlation between these criteria and the GIST-associated proteins such as c-KIT and DOG1 **(Kang *et al.*, 2010).** When we compare the low and the high scores of c-KIT and DOG1with the clinicopathologic parameters of GIST, only the male sex achieved a significant correlation with positive c-KIT expression (P=0.005). In contrast to our results, **Kang *et al.* (2010)** found that c-KIT positivity was associated with female gender (p=0.03). To our knowledge, there is no evidence of association between patient sex and prognosis of GISTs that affects both sexes equally and our results may be an incidental finding related to the random cases selection.

In summary, this study concluded that DOG1 is a better IHC marker than c-KIT in diagnosing GIST due to better sensitivity, NPV and diagnostic accuracy. We recommend using DOG1 as the first choice antibody for the diagnosis of GIST. If it is negative, then c-KIT is tried using 5% as cut off point for its expression. If this latter is also negative and owing to the potential therapeutic significance of GIST diagnosis, mutational analysis should be considered to confirm the diagnosis. It seems that c-KIT and DOG1 are not related to prognosis of GISTs.

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