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Immunohistochemical Study of Dog 1 Protein Expression in Gastrointestinal Stromal Tumors

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Abstract: Gastrointestinal stromal tumors (GISTs) are the most common (80%) mesenchymal tumors of the alimentarycanal, accounting for less than 1% of all gastrointestinal tumors and about 5% all sarcomas with relative incidence in Egypt about 2.5% of all gastrointestinal tumors and 0.3% of all malignancies. They arise from interstitial cells of Cajal (ICC) or their stem cell precursors which are normally are part of the autonomic nervous system of the intestine and serves as a pacemaker function in controlling motility. Molecular pathological studies have shown that most GISTs express C-KIT, a type III receptor tyrosine kinase and have gain of function mutation of the KIT gene or the platelet-derived growth factor receptor alpha (PDGFRA) gene. The identification of KIT (CD117) as a specific immunohistochemical marker, together with the discovery of gain of function mutations in the protooncogene C-KIT in most GISTs represent crucial steps in the definition of this tumor as a biologically distinctive malignancy and, most importantly, in the development of an alternative therapeutic approach using targeted treatment with a specific KIT inhibitors. Approximately 85% to 95% of GISTs are positive for KIT (CD117) by immunohistochemistry. However, about 5% to 15% of GISTs lack KIT expression and are problematic in the diagnosis of GISTs.DOG1 antibody has emerged in recent years as a promising biomarker for GISTs. This study is interested in the immunohistochemical expression of DOG 1 protein in GISTs, in which 40 GISTs were studied using two classic antibodies that are usually used in the diagnosis of GISTs in addition to the DOG 1 antibody. The overall reactivity for both KIT and DOG 1 antibodies were identical (97.5% of cases were positive for each marker with only one case, 2.5%, showed negative staining for each marker), while only 80% of cases were positive for CD 34. These results support the high sensitivity of DOG 1 antibody for diagnosis of GISTs. These results also support KIT as a sensitive mainstay reliable marker for GIST detection and CD 34 as a complementary marker for GIST diagnosis.

Key words: DOG 1 Protein · Gastrointestinal stromal tumors · Immunohistochemical

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common primary mesenchymal tumors of the gastrointestinal tract (GIT) [1]. GISTs are thought to arise from interstitial cells of Cajal (ICC) or their stem cell precursors which are normally part of the autonomic nervous system of the GIT, ICC serves as a pacemaker function in controlling GIT motility [2]. GISTs usually arise in the stomach in 40% to 70%, in the small intestine in 20% to 40% and less than 10% in the esophagus, colon and rectum [3]. GISTs can develop outside the intestinal tract, within the abdomino-pelvic cavity such as the omentum, mesentery and the retroperitoneum; they are called extragastrointestinal stromal tumors (eGISTs), usually behaving aggressively [4]. Molecular pathological studies have shown that most GISTs express KIT, a type III receptor tyrosine kinase and have gain of function mutation of the *KIT* gene or the platelet-derived growth factor receptor alpha (PDGFRA) gene [5]. The identification of KIT (CD117) as a specific immunohistochemical marker, together with the discovery of gain of function mutations in the protooncogene c-kit in most GISTs represent crucial steps in the definition of this tumor as a biologically distinctive malignancy and most importantly in the development of an alternative

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therapeutic approach using targeted treatment with a specific KIT inhibitors [6]. Surgery is the mainstay of therapy; however targeted therapy with imatinib mesylate (a tyrosine kinase inhibitor) has revolutionized therapy of recurrent and metastatic tumors. Sub-classification of GISTs according to kinase mutation status has both biological and clinical implications, since it predicts response to imatinib and prognosis following resection [7]. Approximately 85% to 95% of GISTs are positive for KIT (CD117) by immunohisto chemistry. However, about 5% to 15% of GISTs lack KIT expression and are problematic in the diagnosis of GISTs [5]. DOG1 antibody has emerged in recent years as a promising biomarker for GISTs. It was originally discovered through microarray expression profiling analysis as gene that is highly expressed in GISTs [8]. DOG1 is an antibody against a GIST-specific gene, encoding for the hypothetical protein FLJ10261, dubbed "Discovered on GIST 1" (DOG1), that might play a role in development of GIST and have potential as a diagnostic marker and therapeutic target KIT [9]. DOG1 has shown a high overall sensitivity and specificity for the detection of GISTs. Furthermore, DOG1 immunoreactivity is rarely observed in other mesenchymal and non mesenchymal tumor types. These results support the use of DOG1 as a good diagnostic biomarker for GISTs [8].

The aim of this study is to assess the immunohistochemical expression of DOG1 in Gastrointestinal stromal tumors and correlation with histology and parameters of GIST risk of aggressive behavior. Also to assess the immunohistochemical expression of c-kit (CD 117) and CD34in Gastrointestinal stromal tumors then compare the immunohistochemical expression of DOG1, c-kit (CD 117) and CD 34 and to assess their potential use in diagnosis of gastrointestinal stromal tumors.

MATERIALS AND METHODS

Forty specimens of gastrointestinal stromal tumors have been studied. The specimens were obtained from the Department of Pathology, Cairo university and from other private laboratories, in the period from January 2011 to April 2013.These cases were both retrospective (20 cases) and prospective (20 cases). The specimens were either guided core biopsy specimens (9 specimens) or excisional biopsy specimens (31 specimens). The clinical data and pathological records were retrieved from the files of the patients. The paraffin blocks of all cases were retrieved and four-micron, haematoxylin and eosin-stained sections were generated and reviewed to confirm the diagnoses before inclusion in the study. The slides were revised regarding the; tumor cell morphology (spindle, epithelioid, or mixed) and mitotic rate [expressed as the number of mitotic figures/50 high-power fields (HPFs) in the most mitotic areas; HPF corresponding to 5mm²]. Risk stratification of GISTs (of excision biopsy specimens) considering size and mitotic activity was done following previously published parameters [10]. Immunohistochemical analysis was performed on routinely processed, formalin-fixed, paraffin-embedded tissue. Tissue sections were cut at 3 microns and mounted on poly-L-lysine-coated slides (Super frost slides). Immunohistochemical studies in all cases were performed, using DOG1, c-kit (CD 117) and CD 34. Selected cases (c-kit negative and DOG 1 negative cases) were stained for Actin Smooth Muscle (SMA) and S 100 protein. Positive tissue control is a tissue known to contain the target antigen, e.g. Normal ICC cells and mast cells in the adjacent non neoplastic segment of the GI tract can serve as internal positive control (if present) for c-kit. In contrast to c-kit, monoclonal DOG1antibody stain only the Cajal cells and do not stain mast cells. Negative tissue control is a tissue known not to contain the target antigen e.g. muscle layer negativity for DOG1and c-kit, e.g. The tumor stromal vessels and adjacent normal mucosal and muscularis tissues can serve as good internal negative control. Tissue control for CD 34 was endothelial cells, while nerve fibers served as a control for S-100 protein and muscle fibers as a control for SMA. A colored precipitate at sites of specific cellular antigen localization indicated a positive reaction. DAB gave positive immunostaining which appeared as brownish coloration. The positivity was either diffuse or focal. The results were interpreted in light of the appropriate staining of all positive and negative controls, compared with H&E-stained slides.

C-kit and DOG1 Positivity: DOG 1 and c-kit positivity was either diffuse or patchy and was cytoplasmic, membranous or cytoplasmic with membranous accentuation.

CD 34 Positivity: CD34 positivity was both cytoplasmic and membranous.

S-100 Positivity: S-100 positivity was both cytoplasmic and nuclear.

SMA Positivity: SMA positivity was cytoplasmic.

Each case was scored independently for each tested antibody. According to Kang *et al.* [11], tumors were considered negative when < 10% tumor cells were stained. Cases were considered to be positive only when >10% tumor cells showed unequivocal immunoreactivity. The positive staining intensity of all antibodies was graded as {weakly positive, moderately positive, or strongly positive}, whether the staining was patchy or diffuse.

Statistical Analysis: Statistical analysis was done using the mean, standard error and Chi square test of independence and the Fisher exact test using SPSS V17. A p-value of < 0.05 was considered statistically significant.

RESULTS

The 40 cases of GISTs included in this study were 36 primary tumors (90%), 2 recurrent tumors (5%) and 2 metastatic tumors (hepatic focal lesions) (5%). Out of the 40 cases, 31cases (77.5%) were resection specimens and 9 cases (22.5%) were core biopsies. The cases included 28 men (70%) and 12 women (30%) and the median patient age was 57 years (range 32-77) [with mean age 55.75 and standard deviation ± 10.88]. The median diameter of the resected tumors was 9.8 cm (range 2.2-18 cm) [with mean diameter 10.5 and standard deviation ± 4.55]. The most common location for the tumor was stomach (n=20), followed by small intestine (n=8), omental (n=5), large intestine (n=5) and 2 metastatic cases (HFL) (n=2). Twenty-eight cases (70%) were of spindle cell morphologic type, 8 cases (20%) were epithelioid type and 4 cases (10 %) were of mixed histology. Resected cases were stratified according to tumor risk of progression (relapse) into: high risk, 13 cases (41.94%), intermediate risk 8 cases (25.80%) and low risk 10 cases (32.26%).

The immunoreactions pattern for both c-kit and DOG1 antibody was similar, being observed as cytoplasmic staining with cell membrane accentuation. The prominent cell membrane pattern of staining was mostly observed with DOG1 antibody in cases of epithelioid histology, while the cytoplasmic pattern was mostly observed with KIT antibody, irrespective of the histologic type. CD 34 positivity was both cytoplasmic and membranous and was mostly observed in spindle cell tumors. Of the 40 GIST cases included in this study, 39 cases (97.5%) were positive for DOG1antibody and only one case (2.5%) showed negative staining. The only negative case was a high risk, recurrent, gastric lesion of epithelioid histology. The intensity of positive staining for DOG1 antibody ranged from weakly positive (n=7/39, 17.94%), moderately positive (n=19/39, 48.72%) and strongly positive (n=13/39, 33.34%). No statistically significant relation was found between DOG1 immuno-staining scores and sex of the patient. Correlation of scores to location of tumor also did not show any statistical significance. All spindle cell tumors (n=28/28, 100%) of were positive for DOG1, with the moderate score of staining patterns, being the highest frequency (46.43%), All epithelioid tumors except 1 (n=7, 87.5%) were positive for DOG1 with the moderately positive score representing 50% (n=4/8). There was no statistical significant value in the degree of DOG1 immuno-staining in the different histologic patterns. Epithelioid type histology showed comparable staining intensity results to spindle cell histology showing slightly higher percentage of moderately positive staining (n=4/8, 50%), weakly positive (n=2/8, 25%), strongly positive (n=1/8, 12.5%) and only one negative case (n=1/8, 12.5%). Although the only negative case for DOG 1 antibody was in the high risk group, strong positivity was observed more frequently in this group (6/13,46.15%) in comparison to other risk groups. The results however did not reach statistical significance. Of the 40 GIST cases included in this study, 39 cases (97.5%) were positive for c-kit (CD 117) antibody and only one case (2.5%) showed negative staining.

The only negative case was a high risk, recurrent, large intestinal (rectal) lesion of spindle cell histology. The intensity of positivity staining for c-kit (CD 117) antibody ranged from weakly positive (n=5/39, 12.83%), moderately positive (n=12/39, 30.76%) and strongly positive (n=22/39, 56.41%). No statistically significant relation was found between c-kit (CD 117) immunostaining scores and sex of the patient. Also no correlation was found between c-kit scores and nature of specimen or site of the tumor. Although the only negative case for c-kit (CD 117) antibody was of spindle cell type histology, the greatest percentage of all strongly positive tumors in this study (n=20/39, 51.28%) was observed in spindle cell histologic variant. Twenty out of 28 cases (71.43%) were strongly positive for c-kit in comparison to 1/8 (12.5%) of epithelioid type and 1/4 (25%) of mixed type. The results were statistically significant (P=0.018). Although the only negative case for c-kit (CD 117) antibody was of high risk group, four out of 13 cases (30.77%) of high risk group and 7/13(53.85%) were moderately and strongly positive respectively in

Table 1: Relation between DOG 1& c-kit immunoreactivity for GISTs.

		DOG 1				
c-kit		Negative	Weakly positive	Moderately positive	Strongly positive	Total
Negative	N	0	0	1	0	1
	%	0.00	0.00	100.00	0.00	100.00
Weakly positive	N	0	2	3	0	5
	%	0.00	40.00	60.00	0.00	100.00
Moderately positive	N	1	4	5	2	12
	%	8.33	33.33	41.66	16.66	100.00
Strongly positive	N	0	1	10	11	22
	%	0.00	4.54	45.45	50.00	100.00
Total	N	1	7	19	13	40
	%	2.50	17.50	47.50	32.50	100.00
Kendall's correlation	R	0.436				
	P-value	0.002*				

A highly statistically significant concordance was found between c-kit and CD 34 immunostaining scores (P-value =<0.001*).

Table 2: Relation between c-kit and CD34 antibodies immunoreactivity in studied GISTs.

		C-kit				
CD 34		Negative	Weakly positive	Moderately positive	Strongly positive	Total
Negative	N	1	4	2	1	8
	%	100.00	80.00	16.67	4.55	20.00
Weakly positive	N	0	0	3	3	6
	%	0.00	0.00	25.00	13.64	15.00
Moderately positive	N	0	1	5	8	14
	%	0.00	20.00	41.67	36.36	35.00
Strongly positive	N	0	0	2	10	12
	%	0.00	0.00	16.67	45.45	30.00
Total	N	1	5	12	22	40
	%	100.00	100.00	100.00	100.00	100.00
Kendall's correlation	R	0.493				
	P-value	<0.001*				

Also a statistically significant concordance was found between DOG 1 and CD 34 immunostaining scores (P-value =014).

Table 3: Relation between DOG 1 and CD 34 antibodies immunoreactivity in studied GISTs.

		DOG 1				
CD 34		Negative	Weakly positive	Moderately positive	Strongly positive	Total
Negative	N	1	3	4	0	8
	%	100.00	42.86	21.05	0.00	20.00
Weakly positive	N	0	0	5	1	6
	%	0.00	0.00	26.32	7.69	15.00
Moderately positive	N	0	2	6	6	14
	%	0.00	28.57	31.58	46.15	35.00
Strongly positive	N	0	2	4	6	12
	%	0.00	28.57	21.05	46.15	30.00
Total	N	1	7	19	13	40
	%	100.00	100.00	100.00	100.00	100.00
Kendall's correlation	R	0.342				
	P-value	0.014*				

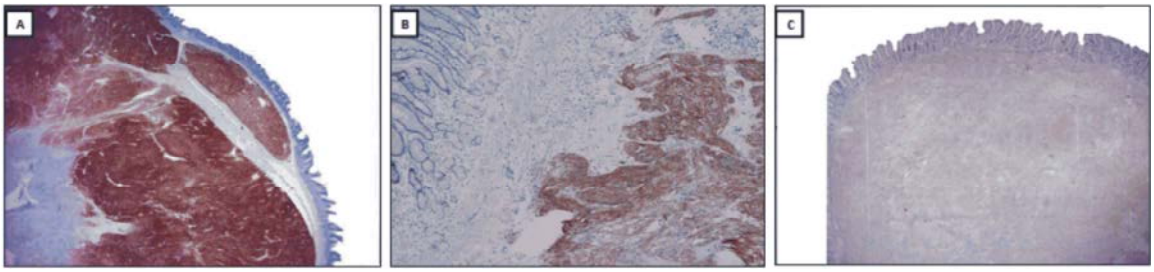

Fig. 1: DOG 1 immuno-reactivity scoring; A-Strongly positive staining, diffuse (x20). B-Moderately positive staining, patchy (x 100). C-Weakly positive staining, patchy (x20).

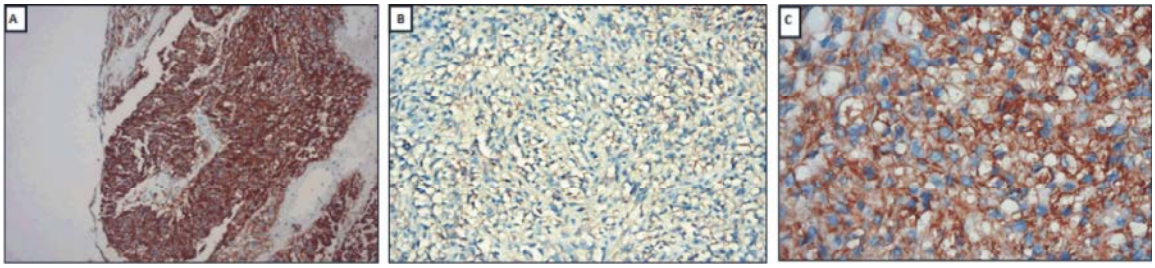

Fig. 2: DOG 1 immunostaining patterns; A-Cytoplasmic staining in spindle cell GIST (x20). B-Membranous staining in epithelioid GIST (x400). C-Cytoplasmic and membranous staining in epithelioid GIST (x 1000).

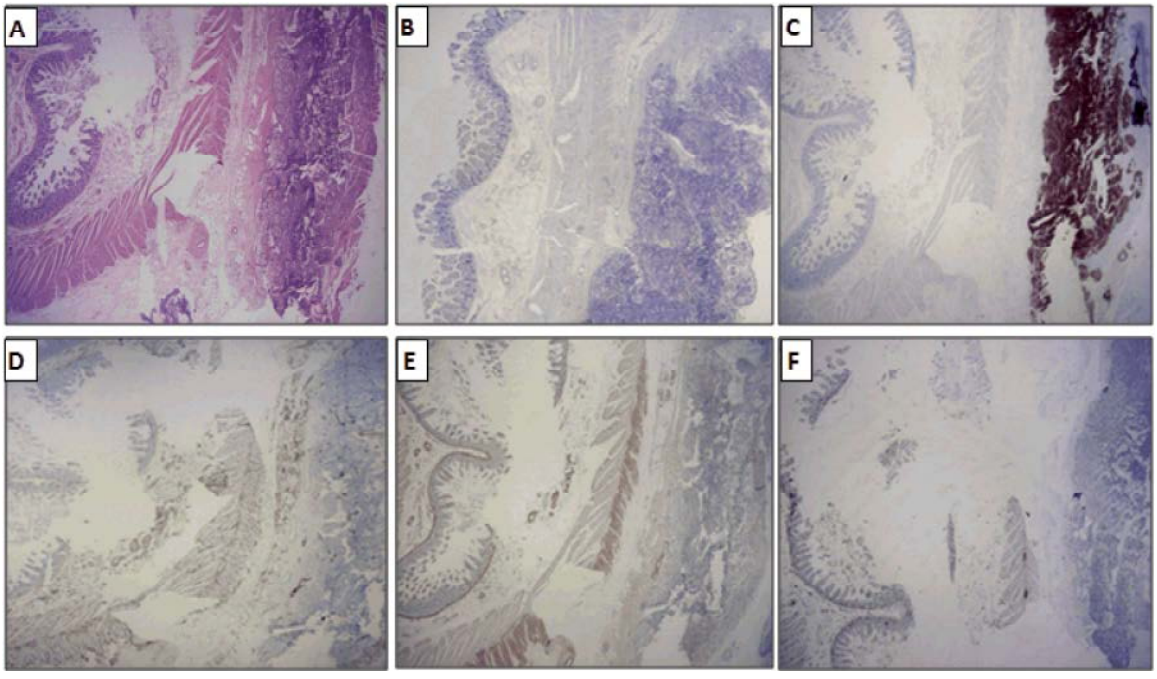

Fig. 3: Gastric spindle cell GIST, A- (H &Ex 20), B- Negative for C-Kit (x20) , C- diffuse strongly positive for DOG 1(x20), D- Negative for CD 34 (x20), E- Negative for SMA (x20), F- Negative for S 100 protein(x20).

comparison to low risk tumors which showed a slightly higher strong positivity score (60%) and nearly similar moderate score (30%) which was statistically insignificant. Of the 40 GIST cases included in this study, 32 cases (80%) were positive for CD 34 antibody and 8 cases (20%) show negative staining. No statistically significant relation was found between CD 34 immunostaining scores and sex of the patient). CD 34 immunoreactivity varied according to site of tumor with significant statistical value (p=0.008).CD 34 immunopositivity was significantly

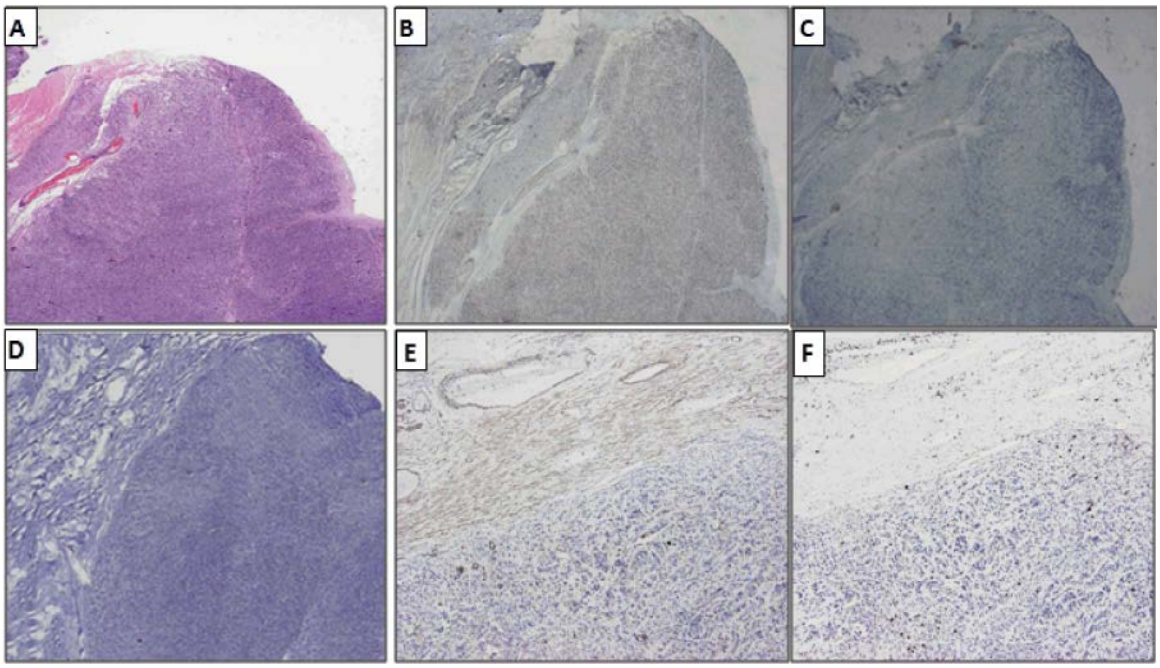

Fig. 4: Epithelioid GIST, A- (H&Ex40), B- Weakly positive for C-Kit (x40) , C- Negative for DOG 1(x40), D- Negative for CD 34 (x40), E- Negative for SMA (x100), F- Negative for S 100 protein (x100).

associated with spindle cell tumors, as 22 out of 28 tumors were positive, Also among positive spindle cell tumors, 39.29% were strongly positive in comparison to only 12.50% strongly positive in epithelioid tumors and 0% in mixed tumors, (p=0.046).

All low risk cases (100%) were negative for CD34 in contrast to intermediate and high risk (25%) and (23.08%) respectively, with results approaching significant value (P- value= 0.072). Of the 40 GIST cases, 38 showed positive immunoreactivity for both c-kit and DOG1 antibodies (95%). One case (2.5%) was c-kit positive, DOG1-negative and conversely the other case was DOG1-positive, c-kit negative. These 2 special cases were stained for both SMA and S100 protein to prove or exclude the possibility of wrong diagnosis of GIST. In each of the two cases both markers showed negative staining of the tumor cells, indicating compatibility with GIST diagnosis. A statistically significant concordance was found between c-kit and DOG1 immunostaining scores (P-value =0.002).

DISCUSSION

In the issue of treatment of GISTs, surgery was and still seizes the upper hand, but before the year 2000, in the pre-imatinib era, there was no effective treatment for metastatic GISTs. The relatively recent development of the tyrosine kinase inhibitors imatinib mesylate and sunitinib malate has revolutionized the treatment of malignant GISTs, but these tyrosine kinase inhibitors are not without side effects and are also expensive treatments [12]. Consequently, accurate specific diagnosis of GISTs enables delivery of potentially life-saving treatment to the right patients. In contrast, selection of the appropriate patient population for this very expensive treatment is part of health care resource optimization [13]. Most GISTs can be identified based on the combination of tumor location, histologic appearance and the presence of KIT by immunohistochemistry [10]. CD117 immunostaining has been a major step forward in the reliable and reproducible diagnosis of GIST, but numerous studies have shown that between 5% and 10% of GISTs fail to immunostaining for CD117, leaving the diagnosis in question, although a large percentage of these tumours still respond to tyrosine kinase inhibitors[14].

Hence, the use of another reliable, available immunohistochemical marker, that is much less expensive than KIT and PDGFRA gene mutation analysis was a necessary to achieve reliable, feasible, rapid and less expensive diagnosis [15]. ‘‘Discovered on GIST-1’’ (DOG1) is a protein encoded by TMEM16A (also known under several names that include TMEM16, FLJ10261, ANO1, ORAOV2 and TAOS2), a gene found to be highly expressed in GISTs by gene-expression profiling [16].

The *DOG1* gene is localized on the chromosome 11 (11q13). It contains 26 exons and encodes for a 960 amino acid protein with an expected size of 114 Kb. On the basis of DNA sequence analysis, the protein has 8 transmembrane domains [17]. In these tumors, DOG1 is strongly expressed on the surface of the neoplastic cells irrespective of mutation status, being rarely expressed in other soft tissue tumors, as demonstrated by earlier studies that used a polyclonal antiserum and even the later developed monoclonal antibody DOG1.1 and clone K9[13]. DOG1 has proved to be very sensitive and specific for the diagnosis of GISTs when compared with C-Kit, including cases of extra-gastrointestinal and metastatic GIST [18]. Schmitt *et al.* [19] found that DOG-1 monoclonal antibody clones (SP31 and K9 clones) yielded the highest sensitivity for GIST. SP31 clone showed a sensitivity of 95% and K9 clone showed a sensitivity of 90%. In contrast, polyclonal and monoclonal c-ki had a sensitivity of 70% and 55%, respectively.

Accordingly, this study was performed in order to test the promising DOG1 immunohistochemical marker, side by side to the use of c-kit (CD 117), the most reliable marker used currently to diagnose GISTs together with use of CD 34, another marker that was previously used to be a GIST reliable immunohistochemical marker. In the present study forty GIST cases were evaluated using the three immunohistochemical markers, with special reference to other used markers (SMA and S100) in special problematic cases. Studied GISTs were found in 28 male patients (70%) and 12 female patients (30%) with male predominance. Thee results are in agreement with those obtained by Kang *et al.* [11],Chan *et al.* [20] and Abdel-Hadi *et al.* [15], who also reported male predominance in their studies, while others, Espinosa *et al.* [8] and Miettinen *et al.* [21] mentioned no sex predominance. In contrast to other studies, especially the European ones, Ahmed *et al.* [22] in United Kingdom, Yan *et al.* [23] in Canada and Monges *et al.* [24] in France and Oddvar *et al.* [25] reported female predominance. Differences between studies likely reflect, among other factors, heterogeneity in GIST coding and reporting to registries across geographic regions.

The median patient age of the patients in current study was 57 years (range 32-77). Similar findings were reported by Mucciarini *et al.* [26] and Tryggvaso *et al.* [27]. Also Nilsson *et al.* [28] reported that GISTs mainly affects middle aged to elderly adults, typically in their 60s. In contrast to other studies, Espinosa *et al.* [8] and Miettinen *et al.* [13], this study didn't include specific clinical groups, such as children and neurofibromatosis type 1 patients mainly due to small sized sampled cases in the current study and also importantly due to lack of registration of such specific groups and lack of specific gene map studies of such cases.

The most common location for the studied tumors was stomach (n=20, 50%), followed by small intestine (n=8, 20%), omental (n=5, 12.5%), large intestine (n=5,12.5%), results that was nearly similar to that found by most of previous studies, Miettinen *et al.* [21], Espinosa *et al.* [8], Miettinen *et al.* [13], Abdel-Hadi *et al.* [15] and Oddvar *et al.* [25].The GISTs histologic subtypes in the current study were found to be Spindle, epithelioid and mixed representing (70%), (20%) and (10 %) respectively. These results are similar to those found by Miettinen *et al.* [29], Fletcher *et al.* [10], Miettinen *et al.* [30] and Foo *et al.* [31].Regarding tumor risk of progression. Based on Fletcher *et al.* [10], the high risk group represented the greatest percentage, followed by low risk and lastly the intermediate risk group being represented as (41.94%), (32.26%) and (25.80%), respectively. These results are nearly similar to those found by Jung *et al.* [32] being (43.2%) and (30.9%) and (19.8%) for high risk, low risk and intermediate risk groups respectively, with addition of the very low risk group (6.1%). While, others found different results regarding the low risk groups (low and very low groups), found by Kang *et al.* [11] [35% - high risk, 8% - intermediate risk, 15%-low risk and 42%-very low risk] and Oddvar *et al.* [25].[High risk-31%, intermediate risk-19%, low risk-22% and very low risk-28%]. These findings of reporting high percentage of low risk groups in those studies are probably due to early detection of such cases and advanced methods of diagnosis which allow early detection of the tumors even if very small sized, which is not always available in our studied cases. West *et al.* [16], first reported DOG1 to be of superior sensitivity and specificity to KIT using DOG1 being expressed in 97.8% (136/139) studied GISTs (using polyclonal antibody). With the generation of subsequent developed monoclonal antibodies against DOG1 (DOG1.1 & DOG1.3), *Espinosa et al.* [8] demonstrated similarly superior sensitivity and specificity of these antibodies compared with KIT, DOG1 reactivity was seen in 87% of GIST cases, whereas the expression of KIT was found in 74%. Later on *Miettinen et al.* [13] used another developed K9 monoclonal antibody and found DOG1-positivity in a great majority of GISTs of all sites, (94.4% for DOG1 and 94.7% for c-kit). These results are similar to the current study [using the monoclonal antibody (SP 31)] in which both c-kit and DOG 1 antibodies showed

immunoreactivity being (97.5%) for each marker with identical sensitivity for both markers. Also similar results were found by Abdel-Hadi *et al.* [15], who found a remarkable concordance between the results of KIT and DOG1.1, as 93.6% of GISTs were positive for both markers compared with the current study (95.5%) for both markers.

In the current study, the intensity of positive staining for DOG 1 antibody ranged from weakly positive (17.94%), moderately positive (48.72%) and strongly positive (33.34%). Kang *et al.* [11], also found that the overall staining intensity for DOG 1 was weak (21%), moderate (34%) and strong (36%), results that are relatively similar to our study. In the current study, the only negative DOG1 case was a high risk, recurrent, gastric lesion of epithelioid histology. This result is in agreement with the findings of Jung *et al.* [32], who stated that DOG1-negative GIST cases were significantly correlated with recurrence and/or metastasis. Also, Miettinen *et al.* [13] stated that some gastric epithelioid GISTs were KIT-positive and DOG- negative, although the gastric epithelioid GISTs were more consistently positive for DOG1. In spite of those Espinosa *et al.* [8], reported that DOG1 expression was not related to the type of mutation (*KIT or PDGFRA*), site, tumor size, tumor grade, or patient age. The only negative c-kit (CD117) case was a high risk, recurrent, large intestinal (rectal) lesion of spindle cell histology. This result was coincides with Kang *et al.* [11], who reported that most c-kit negative GIST were mostly located in the stomach (96%) and in the rectum (4%). The histological subtype was spindle in (46%), epithelioid in (42%) and mixed in (12%). On the basis of tumor locations, sizes and mitotic counts, (35%) of GISTs were classified as high risk, (8%) as intermediate risk, (15%) as low risk and (42%) as being at a very low risk of aggressive behavior.

It is worth noting that, concordant percentage findings between current study and previously mentioned studies of Espinosa *et al.* [8], Abdel-Hadi *et al.* [15] and Miettinen *et al.* [13], regarding GISTs with c-kit positive/ DOG1 negative pattern and GISTs with c-kit negative/DOG1 positive pattern, recommend the combination of using both antibodies in diagnosis and reassuring suspected GIST cases. Considering CD 34 positive staining as a previously reliable immuno-histochemical marker for GIST diagnosis and currently as a secondary or complementary marker, current study showed (22/40, 80%) in agreement with most of previous studies, (80%) by Miettinen *et al.* [2], (64%) by Espinosa *et al.* [8], (80%) by Liegl *et al.* [33], (81%) by Novelli *et al.* [34] and (76%) by Kang *et al.* [11]. Gathering the previous data, none of the tested markers had a diagnostic accuracy of 100% positivity (97.5 % positivity for each of c-kit and DOG1 and 80% positivity for CD 34). Hence, the use of DOG 1 in clinical practice as either a complementary to c-kit or as a part of a panel can allow the identification of more GIST cases that can benefit from the effective tyrosine kinase inhibitors.

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