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Overview of MDM2 and B-RAF Expression in Gastric Lesions

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Abstract

BACKGROUND: Globally, gastric cancer (GC) it is the fourth most common cancer and the third cause of cancer-related deaths. Overexpression of MDM2 and B-RAF appeared to be increased in malignancy and associated with poor prognosis in several human tumours, but their role in gastric cancer remains controversial.

AIM: We had investigated the immunohistochemical expression of MDM2 and B-RAF in 136 gastric lesions with/without *H. pylori* association.

MATERIAL AND METHODS: Studied specimens include chronic gastritis (32), intestinal type GC (70), diffuse GC (22) and gastrointestinal stromal tumours (GIST) (12).

RESULTS: MDM2 expression increased significantly in intestinal GC compared to other groups ($p < 0.001$), while B-RAF expression increased significantly in GIST compared to other groups ($p < 0.001$). *H. pylori* increased expression of MDM2 in intestinal GC cases but did not affect B-RAF expression. MDM2 expression correlated with high grade of tumor differentiation ($p < 0.001$), deep invasion ($p < 0.05$), nodal metastases ($p < 0.05$) and distant metastases ($p < 0.1$) in intestinal GC, while B-RAF expression did not correlate with TNM stage ($p < 0.1$).

CONCLUSION: MDM2 up-regulation was more frequent in intestinal GC, while B-RAF up-regulation was more frequent in GIST compared to other groups; MDM2 expression in intestinal GC was correlated with *H. pylori* association, high grade of differentiation, deep invasion, nodal and distant metastases, meanwhile, B-RAF expression was correlated with high-grade intestinal GC but did not correlate with *H. pylori* or TNM stage. The possible role of both MDM2 and B-RAF in predicting progression of gastric tumours and prognosis deserves further investigations.

Introduction

Worldwide, Gastric cancer (GC) is the fourth most common cancer in men (8.5%) and the third cause of cancer-related deaths (10.1%). In the female, it is the fourth most common cancer (4.8%) and the third cause of cancer-related deaths (7.2%) [1]. Although the incidence of gastric cancer has gradually decreased over the last half-century, the prognosis of advanced gastric cancer remains poor and gastric cancer-related mortality rates remain unacceptable in many areas [2]**.**

Gastric carcinogenesis is a multistep and multifactorial process. The intestinal type of gastric cancer is often related to environmental factors such as Helicobacter pylori infection, diet, and lifestyle, while the diffuse type is more often associated with genetic abnormalities [3].

The *Helicobacter pylori* (*H. pylori*) bacterium is responsible for 5.5% of all infection-associated cancers and is the major cause of gastric cancer in consequence of chronic inflammation [4]. Persistent gastric mucosa inflammation results in chronic gastritis and progresses through a multistep process to gastric atrophy, intestinal metaplasia, dysplasia, and finally carcinoma [5].

In Egypt, infection with *H. pylori* is common, and acquisition of infection occurs at a very young age [6]. Also, gastric cancer is the 13th most common cancer in men (1.8%) and the 10th cause of cancer-related deaths (2.2%). In the female, it is the 14th most

common cancer (1.5%) and the 11th cause of cancer-related deaths (2.2%). For both sexes, it is the 12th most common cancer (1.6%) and 11th cause of cancer-related deaths (2.2%) [7].

Several biological markers are tested as potential predictors of the gastric carcinoma outcome, and some of them are essential to developing a malignancy. *MDM2* (Murine double minute 2) is an oncogene that has been mapped to chromosome 12q13–14 and encodes a 90 kDa cellular oncoprotein. The gene structure on the human chromosome was identified in 1992 [8]. It binds to, and negatively regulates, transactivation of p53 and was then itself found to be a transcriptional target of p53, defining a negative feedback loop of p53 tumour suppressor gene [9]. The *MDM2* oncogene played an important role in cancer progression as overexpression of *MDM2* in tumour cells induced cell proliferation and inhibits cell apoptosis [10]. Several studies have shown that *MDM2* overexpression was associated with poor survival and was a useful predictive factor for poor prognosis in humans with hepatocellular carcinoma and breast carcinomas [11] [12].

V-RAF murine sarcoma viral oncogene homolog B1 (*B-RAF*) is a member of the RAF family of protein kinases which has three members: *A-RAF*, *B-RAF* and *Raf-1* [13]. All RAF proteins are serine/threonine kinases located in the RAS/RAF/MEK/ERK cascade as downstream effectors of RAS and can phosphorylate and activate MEK, which in turn activates ERK. *B-RAF* is the most potent activator of MEK [14] [15] and is the only one known to be activated by mutation in human cancer [16]. They are mainly found in melanoma, thyroid papillary carcinoma and colorectal tumours with microsatellite instability [17].

In this study, we investigated immunohistochemical expression of *MDM2* and *B-RAF* in chronic gastritis and malignant gastric lesions; and their correlation with *H. pylori* association, tumour location, grade, and TNM stage in Egyptian patients.

Material and Methods

This study was conducted on 136 archival gastric paraffin blocks from Pathology Department of Theodor Bilharz Research Institute. All samples had been obtained as endoscopic biopsies or gastrectomy specimens. The study protocol was approved by the Ethics committee of Theodor Bilharz Research Institute, for the protection of human subject and adopted by the 18th world medical assembly, Helsinki, Finland (2013).

Our studied lesions were classified into four groups: chronic gastritis: 32 specimens; intestinal GC: 70 specimens; diffuse GC: 22 specimens; GIST: 12 specimens.

Gastric tissue sections were stained by Hematoxylin-eosin for routine diagnosis, grading and staging of tumours. Giemsa stain was used to detect *H. pylori* in gastric sections.

Immunohistochemistry for *MDM2* and *B-RAF* was performed on tissue sections cut from the paraffin blocks at 4µm onto positively charged slides (Superfrost Plus, Menzel-Glaser, Germany) and stained on an automated platform (Dako Autostainer Link 48) using: anti-human *MDM2* monoclonal primary antibodies (Clone MSP14, NeoMarkers, Fremont, CA, USA) and anti-B-RAF pV600E (Spring Bioscience, Pleasanton, CA; purchased from Zytomed Systems, Berlin, Germany) at 1:200 dilution. Heat-induced antigen retrieval was used for 30 min at 97°C in the high-PH EnVision™ FLEX Target Retrieval Solution.

For each setting, positive and negative control slides were included. As a negative control, gastric tissue was processed, but the primary antibodies were not added and instead add non-immune immunoglobulin G (IgG; DAKO, Glostrup, Copenhagen, Denmark). The positive control was a section of liposarcoma for *MDM2* and colorectal carcinoma for *B-RAF*.

All sections were assessed and scored. The sections were examined by using light microscope [Scope A1, Axio, Zeiss, Germany]. Photomicrographs were taken using a microscope-camera [AxioCam, MRc5, Zeiss, Germany]. All procedures were done at the pathology department of Theodor Bilharz Research Institute, Cairo, Egypt.

Scoring of *MDM2* immunostaining was performed semiquantitatively, using digital images and 22-in monitor with hardware calibration capabilities. Staining was considered to be negative (0) if no staining was seen within a tumour, weakly positive (1+) if focal staining was seen, and strongly positive (2+) if there was diffuse staining in more than 80% of tumour cells [18]. Nuclear staining could be detected in very few cases, and the vast majority of positive cases showed only cytoplasmic staining.

The intensity of cytoplasmic immunostaining was scored from zero to 3 (0: no staining, 1: weak, 2: moderate and 3: strong) [19]. Cases with moderate and strong immunostaining were considered positive [20].

We have also counted the percentage of cells with positive expression in 5 successive high power fields.

The immunohistochemical results were analysed using SPSS version 20 (IBM Corporation, Armonk, New York, USA). Data are presented as the mean ± S.D. Two-tailed Student's *t*-tests and one-way

ANOVA were used to evaluate the data. Comparison of difference in percentage between groups was evaluated using two-tailed Fischer's exact test. Differences were considered statistically significant at $P < 0.05$.

Results

Different studied gastric lesions were more common in males (73.5%) than females (26.5%). The differences were statistically significant ($p < 0.05$) in cases of chronic gastritis and intestinal GC, while non-significant in cases of diffuse GC and GIST ($p > 0.05$) (Table 1).

Table 1: Gender in different studied lesions

Lesion	Gender		Total no. (%)
	Female no. (%)	Male no. (%)	
Chronic gastritis	4_a (11)	28_b (28)	32 (23.5)
Intestinal GC	24_a (66.7)	46_b (46)	70 (51.5)
Diffuse GC	6_a (16.7)	16a (16)	22 (16.2)
GIST	2_a (5.6)	10a (10)	12 (8.8)
Total	36	100	136

GC: gastric cancer, GIST: gastrointestinal stromal tumor.

Endoscopically, cases of chronic gastritis represented usually as diffuse mucosal lesions, cases of intestinal and diffuse GC represented as fungating or ulcerative lesions and usually located at the gastro-oesophagal junction (GEJ) or pylorus, while GIST cases represented as mass lesions. No significant differences were found considering endoscopic appearance or location of studied gastric lesions (Table 2).

Table 2: Endoscopic appearance and location of studied gastric lesions

		Lesion				Total
		Chronic gastritis no. (%)	Intestinal GC no. (%)	Diffuse GC no. (%)	GIST no. (%)	
Endoscopic appearance	Diffuse	32_a (100)	0_b	0_b	0_b	32 (23.5)
	Fungating	0_a	64_b (91.4)	20_b (90.9)	0_a	84 (61.8)
	Mass	0_a	0_a	0_a	8_b (66.7)	8 (5.9)
	Ulcer	0_a	6_a (8.6)	2_a (9.1)	0_a	8 (5.9)
	Wall thickening	0_a	0_a	0_a	4_b (33.3)	4 (2.9)
	Unavailable	32_a (100)	$56_{b,c}$ (80)	14_c (63.6)	$12_{a,b}$(100)	114(83.8)
Anatomic site	Cardia	0_a	2_a(2.9)	0_a	0_a	2(1.5)
	Diffuse	0_a	2_a(2.9)	0_a	0_a	2(1.5)
	Fundus	0_a	4_a(5.7)	0_a	0_a	4(2.9)
	GEJ	0_a	$4_{a,b}$(5.7)	4_b(18.2)	$0_{a,b}$	8(5.9)
	Pylorus	0_a	2_a(2.9)	4_a(18.2)	0_a	6(4.4)
Total		32	70	22	12	136

GC: gastric cancer, GEJ: gastro-esophageal junction, GIST: gastrointestinal stromal tumor.

Cases of intestinal GC and diffuse GC showed the significantly higher percentage of *H. pylori* positivity compared to chronic gastritis and GIST ($p < 0.05$) (Table 3).

All studied chronic gastritis and GIST cases were negative for *MDM2* expression. *MDM2* positivity was identified in 31.4% of intestinal GC and 9.1% of diffuse GC, with the statistically significant difference between intestinal GC and other groups ($p < 0.001$) as well as between diffuse GC and both chronic gastritis and GIST ($p < 0.05$).

Table 3: Association between *H. pylori* and different studied lesions

H. pylori	Lesion				Total no. (%)
	Chronic gastritis no. (%)	Intestinal GC no. (%)	Diffuse GC no. (%)	GIST no. (%)	
Positive	12_a (37.5)	44_b (62.9)	$14_{a,b}$(63.6)	$6_{a,b}$ (50)	76 (55.9)
Negative	20_a (62.5)	26_b (37.1)	$8_{a,b}$ (36.4)	$6_{a,b}$ (50)	60 (44.1)
total	32	70	22	12	136

GC: gastric cancer, GIST: gastrointestinal stromal tumor.

On the other hand, *B-RAF* positivity was identified in all studied GIST cases, 22.9% of intestinal GC and 6.2% of chronic gastritis cases, while all diffuse GC were negative, with statistically significant difference comparing GIST to other groups ($p < 0.001$) and comparing intestinal GC to chronic gastritis and diffuse GC ($p < 0.05$) (Table 4).

Table 4: *MDM2* and *B-RAF* immunoreactivity in different lesions

Lesion	*MDM2*		*B-RAF*		Total
	Negative no. (%)	Positive no. (%)	Negative no. (%)	Positive no. (%)	
Chronic gastritis	32 (100)	0	30 (93.8)	2	32
Intestinal GC	48 (68.6)	22 (31.4)**	54 (77.1)	16 (22.9)#	70
Diffuse GC	20 (90.9)	2 (9.1)*	22 (100)	0	22
GIST	12 (100)	0	0	12 (100)**	12
Total	112	24	106	30	136

GC: gastric cancer, GIST: GIST: gastrointestinal stromal tumor; ** Significant difference with other groups ($p < 0.001$); * Significant difference with chronic gastritis and GIST ($p < 0.05$); #Significant difference with chronic gastritis and diffuse GC ($p < 0.05$).

Mean percentage of *MDM2* positive cells and intensity of expression were significantly higher in intestinal GC followed by diffuse GC compared to chronic gastritis and GIST cases ($p < 0.001$), while mean percentage of *B-RAF* positive cells and the intensity of expression were significantly higher in GIST followed by intestinal GC compared to chronic gastritis and diffuse GC cases ($p < 0.001$) (Table 5).

Table 5: Expression of *MDM2* and *B-RAF* (mean percentage of positive cells and intensity of expression) in different studied lesions

Lesion (no.)	*Mdm2*		*B-raf*	
	Percent Mean ± Std. Error of mean	Intensity Mean ± Std. Error of mean	Percent Mean ± Std. Error of mean	Intensity Mean ± Std. Error of mean
Chronic gastritis (32)	0.50 ± 0.35	0.06 ± 0.04	2.31 ± 1.32	0.19 ± 0.09
Intestinal GC (70)	8.51 ± 1.28	0.94 ± 0.08	15.49 ± 3.12	0.74 ± 0.10
Diffuse GC (22)	1.45 ± 0.70	0.27 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
GIST (12)	0.00 ± 0.00	0.00 ± 0.00	86.67 ± 1.42	2.67 ± 0.14
p value	P < 0.001	P < 0.001	P < 0.001	P < 0.001

GC: gastric cancer, GIST: GIST: gastrointestinal stromal tumor.

For statistical purposes, we separately studied the relation between clinic-pathological features of intestinal GC cases and immunohistochemical expression results of *MDM2* and *B-RAF*.

As regards the endoscopic appearance of intestinal GC; fungating lesions exhibited a higher percentage of *MDM2* positive cells and *MDM2* intensity of expression, while ulcerative lesions

exhibited a higher percentage of *B-RAF* positive cells and *B-RAF* intensity of expression. However, these relations did not reach a significant difference between examined groups (p > 0.1) (Table 6).

Table 6: Relationship between the expression of MDM2 and B-RAF with the Endoscopic appearance of intestinal GC

Endoscopic appearance (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean ± Std. Error of mean	
Fungating (64)	8.94 ± 1.38	0.97 ± 0.08	15.38 ± 3.33	0.72 ± 0.10
Ulcer (6)	4.00 ± 1.26	0.67 ± 0.21	16.67 ± 9.01	1.00 ± 0.37
P value	P > 0.1	P > 0.1	P > 0.1	P > 0.1

Considering the tumour location, the mean percentage of *MDM2* positive cells and intensity of expression were significantly higher in tumours with the diffuse location, followed by GEJ compared to other sites (p < 0.001). On the other hand, **the** mean percentage of *B-RAF* positive cells and intensity of expression were higher in tumours at GEJ followed by fundus compared to other sites; the difference was statistically significant for *B-RAF* intensity score (p < 0.001) but non-significant for *B-RAF* per cent (p > 0.1) (Table 7).

Table 7: Relationship between the expression of MDM2 and B-RAF with anatomical site of intestinal GC

Anatomical site (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean± Std. Error of mean	
Undefined (56)	5.18 ± 0.50	0.79 ± 0.06	14.71 ± 3.51	0.68 ± 0.10
Cardia (2)	5.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Diffuse (2)	40.00 ± 0.00	3.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fundus (4)	17.50 ± 7.22	1.00 ± 0.00	20.00 ± 11.58	1.00 ± 0.58
GEJ (4)	29.00 ± 12.12	2.00 ± 0.58	45.00 ± 1443	2.50 ± 0.29
Pylorus (2)	15.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P value	P < 0.001	P < 0.001	P > 0.1	P < 0.001

GEJ: gastro-oesophageal junction.

Regarding *H. pylori* association, the mean percentage of *MDM2* positive cells and intensity of expression were higher in *H. pylori*-associated intestinal GC compared to *H. pylori* non-associated tumours, without a statistically significant difference (p > 0.1). On the contrary, mean percentage of *B-RAF* positive cells and intensity of expression were higher in *H. pylori* non-associated intestinal GC, without statistical significance (p > 0.1) (Table 8).

Table 8: Relationship between the expression of MDM2 and B-RAF with H. pylori association of intestinal GC

H. Pylori (no. Of lesions)	*Mdm2*		*B-raf*	
	Percent	Intensity	Percent	Intensity
	Mean ± std.error of mean		Mean ± std.error of mean	
Positive (44)	9.41 ± 1.85	1.00 ± 0.11	14.55 ± 3.87	0.73 ± 0.13
Negative (26)	7.00 ± 1	0.85 ± 0.07	17.08 ± 5.36	0.77 ± 0.14
P value	P > 0.1	P > 0.1	P > 0.1	P > 0.1

Mean percentage of *MDM2* positive cells and intensity of expression were significantly higher in high grade intestinal GC compared to low grade ones (p < 0.0001 & p < 0.01 respectively), and in high stage compared to lower stages; the difference was statistically significant for *MDM2* intensity score (p < 0.05) and non-significant for *MDM2* percent (p > 0.05), additionally percentage of *MDM2* positive cells and intensity of expression increased significantly with increasing lymph node stage (p < 0.05 and < 0.0001 respectively) and with presence of distant metastases; the difference was statistically significant for *MDM2* intensity score (p < 0.05) and non-significant for *MDM2* percent (p > 0.01) (Table 10).

Table 9: Relationship between the expression of *MDM2* and *B-RAF* with intestinal GC grade of differentiation

Grade (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean ± Std. Error of mean	
High (12)	21.83 ± 5.77	1.50 ± 0.34	25.83 ± 8.28	1.17 ± 0.37
Low (58)	5.76 ± 0.52	0.83 ± 0.05	13.34 ± 3.33	0.66 ± 0.09
P value	P < 0.0001	P < 0.01	P > 0.1	P < 0.05

In addition, mean percentage of *B-RAF* positive cells and the intensity of expression were higher in high-grade intestinal GC compared to low-grade tumours; the difference was statistically significant for *B-RAF* intensity score (p < 0.05) and non-significant for *B-RAF* per cent (p > 0.1) (Table 9), moreover, these parameters were higher in T3 intestinal GC compared to T2 and T4 without statistical significance (p > 0.1) (Table 8). Also, *B-RAF* parameters were higher in N1 stage compared to N0 and N3 and in M0 compared to M1without statistical significance (Table 10).

Table 10: Relationship between the expression of MDM2 and B-RAF in intestinal GC with TNM stage

Item (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean ± Std. Error of mean	
T				
2 (12)	2.50 ± 0.75	0.50 ± 0.15	13.83 ± 7.62	0.67 ± 0.22
3 (38)	9.21 ± 2.08	1.00 ± 0.12	18.84 ± 4.85	0.79 ± 0.13
4 (20)	10.80 ± 1.76	1.00 ± 0.00	10.10 ± 3.76	0.70 ± 0.18
P value	P > 0.05	P < 0.05	P > 0.1	P > 0.1
N				
0 (28)	5.14 ± 1.09	0.57 ± 0.10	7.07 ± 3.40	0.50 ± 0.12
1 (26)	8.15 ± 1.29	1.00 ± 0.00	22.46 ± 5.67	0.92 ± 0.15
3 (16)	15.00 ± 4.52	1.50 ± 0.22	18.88 ± 7.64	0.88 ± 0.27
P value	P < 0.05	P < 0.0001	P > 0.05	P > 0.1
M				
0 (52)	8.04 ± 1.47	0.85 ± 0.08	17.00 ± 3.67	0.85 ± 0.12
1 (18)	9.89 ± 2.60	1.22 ± 0.15	11.11 ± 5.95	0.44 ± 0.12
P value	P > 0.1	P < 0.05	P > 0.1	P > 0.05

Each subscript letter denotes a subset of gender categories whose column proportions do not differ significantly from each other at the 0.05 level.

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Discussion

Gastric cancer is still a serious public health problem in the world. The high mortality rate that is seen globally is mainly due to the advanced stage at

diagnosis with the availability of few biomarkers for early detection [21].

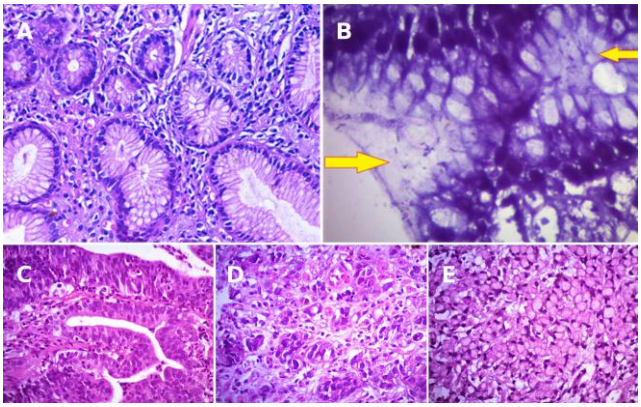

Figure 1: Sections from gastric tissue showing: A) A case of chronic gastritis (H&E stain X200); B) Helicobacter pylori microorganisms in relation to surface epithelium of gastric mucosa (arrows) (Giemsa stain X 400); C) A case of intestinal type gastric adenocarcinoma of low grade (H & E stain X 200); D) A case of high-grade gastric adenocarcinoma; intestinal type (H & E stain, X 200); E) A case of diffuse gastric carcinoma of signet-ring type (H & E stain X 200)

In the present work, male predominance was reported which is similar to the worldwide trend (2:1) [22], as 73.5% of gastric lesions belonged to males compared to 26% belonged to females, with incidence 2.8:1. A percentage lower than ours reported by Gaballah et al., [23] and Darwish et al., [24] who reported male to female ratio of 1.2:1 and 1.3: 1 respectively.

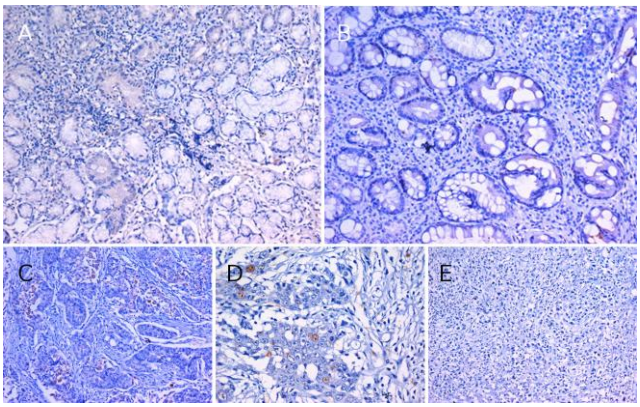

Figure 2: IHC using anti-MDM-2 monoclonal antibody in gastric sections: A) A case of chronic gastritis negative for MDM2 expression (X 200); B) A case of chronic gastritis with intestinal metaplasia negative for MDM2 (X 200); C) Sections in intestinal-type gastric carcinoma, low grade, showing mild focal nuclear expression of MDM2 (X 100); D) Section in intestinal-type gastric carcinoma, high grade, showing mild focal expression of MDM2 (X 200); E) Section in signet-ring type gastric carcinoma, negative for MDM2 expression (X 100)

The International Agency for Research on Cancer (IARC) classified *H. pylori* bacterium as a Group I carcinogen [25] *H. pylori is* a pathogen that colonises the gastric epithelium and causes chronic inflammation and considerably increases the risk of developing GC [26]**.** Our study showed that *H. pylori* were significantly associated with intestinal-type and diffuse GCs compared to GISTs and chronic gastritis, this comes by previous reports [27] [28] [29].

Endoscopically, our studied data sheet showed that cases of chronic gastritis usually represented as diffuse mucosal lesions, cases of intestinal and diffuse GC represented as fungating or ulcerative lesions, while GIST cases represented as mass lesions. Anatomically, no significant difference was detected considering the location of studied gastric lesions. Anatomical site of most of our studied lesions had not been mentioned. However, GEJ was the most frequent site mentioned for GCs; and this could be related to gastro-oesophageal reflux.

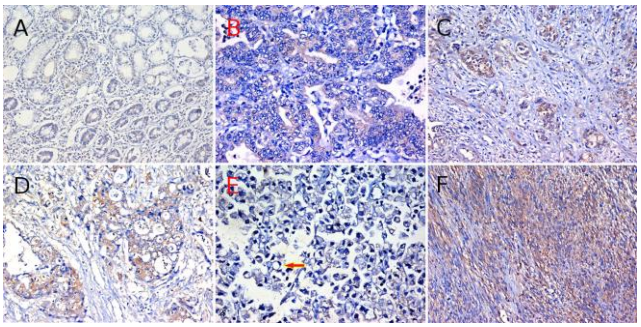

Figure (3): IHC using anti-B-RAF monoclonal antibody in gastric sections expressed as brown cytoplasmic staining (X 200): A) A case of chronic gastritis showing negative B-RAF expression (X 200); B) A case of intestinal type gastric adenocarcinoma, low grade, showing mild focal B-RAF expression (X 200); C) & D) Sections in intestinal-type gastric adenocarcinoma, high grade, showing moderate B-RAF expression (X 200); E) A case of diffuse gastric carcinoma (signet ring pattern) showing negative B-RAF expression (X 200); F) A case of gastrointestinal stromal tumour (GIST) showing moderate B-RAF expression (X200)

Wade et al., [30] and Li and Lozano [10] reported that *MDM2* oncogene played an important role in cancer progression and *MDM2* overexpression in tumour cells induced cell proliferation inhibited cell apoptosis. We found *MDM2* positivity in 31.4% of intestinal GC cases. Gunther et al., [31] found *MDM2* expression in 45% of intestinal GCs. However, Ye et al., [32] reported a much higher per cent, as they detected *MDM2* immunopositivity in 70.4% of their GC cases. Moreover, intestinal GC exhibited a significantly higher percentage of *MDM2* positive cells (8.51%) and higher intensity of expression compared to other groups. This matches the findings of Gunther et al., [31] and Nakajima [33] who detected *MDM2* positivity in 10% and 7.76% of gastric cancer cells respectively. Shen et al., [34] stated that *MDM2* expressed at higher levels in GC tissues than in non-cancerous gastric mucosa. On the contrary, Busuttil et al., [21] observed negligible levels of *MDM2* staining in GC samples. Variable results between studies may be attributed to different risk factors promoting to gastric cancer including *H. pylori*, obesity, tobacco smoking, red meat, a high-salt diet, alcohol, and low socioeconomic status, genetic polymorphisms, the age of cancer onset and gender.

On the other hand, *B-RAF* was expressed in

all GIST specimens that showed a significantly higher mean percentage of *B-RAF* positive cells (86.67%) and higher intensity of expression compared to other groups. This matches with findings of Holstein et al., [35] who observed *B-RAF* expression in all GIST cases in more than 80% of cells. On the contrary, several other studies reported a much smaller percentage of *B-RAF* positivity in GIST than ours [36] [37] [38] as they detected *B-RAF* mutation in 7%, 3.8% and 3.5% of GISTs respectively. Furthermore, intestinal GC cases showed significantly higher expression of *B-RAF* (higher number of positive cases, the percentage of positive cells and intensity of expression) compared to chronic gastritis and diffuse GC. Many previous studies reported the presence of a *B-RAF* mutation in patients with gastric adenocarcinoma [27] [39] [40].

Considering cases of intestinal type GC, no statistically significant difference was achieved when comparing fungating and ulcerating intestinal GC for parameters of *MDM2* and *B-RAF* expression (mean percentage of positive cells and intensity of expression). Tumours with diffuse location and at GEJ showed significantly higher mean percentage of *MDM2* positive cells and *MDM2* intensity of expression. On the other hand, tumours at GEJ and fundus showed non-significantly higher mean percentage of *B-RAF* positive cells and significantly higher *B-RAF* intensity of expression. To our knowledge, no other studies demonstrated *MDM2* or *B-RAF* expression about endoscopic appearance or anatomical site of intestinal GC.

In the present study, *MDM2* parameters were non-significantly higher in *H. pylori*-associated intestinal GC than in *H. pylori* non-associated ones. This goes with many previous studies reporting that *H. pylori* infection was associated with higher expression of *MDM2* in intestinal metaplasia and gastric carcinoma [33] [41] [42]. Furthermore, Kodama et al., [43] reported that successful eradication of *H. pylori* dramatically reduced *MDM2* levels. On the contrary, *B-RAF* parameters were non-significantly higher in *H. pylori*-non-associated intestinal GC than in *H. pylori*-associated ones; however, Sabry et al., [27] found a significant positive relationship between the qPCR of *H. pylori* and quantitative *B-RAF* in GC cases.

As regards different grades of differentiation in intestinal GC, we found a statistically significant higher percentage of *MDM2* positive cells and non-significant higher percentage of *B-RAF* positive cells in high-grade tumours compared to low-grade ones. This goes with findings of Sabry et al., [27] who detected a significant positive correlation between grades of GC and qPCR of *B-RAF*.

Our current results showed an increase in *MDM2* expression parameters with increasing depth of invasion, the presence of distant metastases and lymph node metastases. This matches with Ye et al., [32] results which reported that *MDM2* expression was associated with depth of invasion, lymph node metastases and distant metastases. Sepideh et al., [44] found a direct correlation between lymph node metastases and *MDM2* staining intensity; meanwhile, they did not find a remarkable correlation between *MDM2* expression and nodal involvement.

As regards *B-RAF* expression parameters in intestinal GC, no significant differences were achieved with different tumour stages, different stages of lymph node metastasis and state of distant metastases. These findings match results of other previous studies which did not find a relationship between *B-RAF* expression and histopathological variables of GC [45] [46] [47].

In conclusion, we found that: (1) *MDM2* up-regulation was more frequent in intestinal GC compared to other groups, while *B-RAF* up-regulation was more frequent in GIST compared to other groups; (2) *H. pylori* induces *MDM2* up-regulation in intestinal GC; (3) In intestinal GC cases, *MDM2* expression was correlated with high grade of differentiation, deep invasion, nodal and distant metastases, meanwhile, *B-RAF* expression was correlated with high-grade tumours but had no association with TNM stage. The possible role of both *MDM2 and B-RAF* in predicting progression of gastric tumours and prognosis deserves further investigations.

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