

Original Research Article

Microsatellite Instability and P53 Aberrant Expression in Gastric Adenocarcinoma: Immunohistochemical Assessment and Correlation with Clinicopathological Characteristics

Abstract

Background: Gastric cancer (GC) is among the five most frequent cancer worldwide, following lung, breast, colorectal, and prostate cancer. It is the 12th most prevalent cancer in both sexes in Egypt, accounting for 1.6% of all malignancies. Distinct molecular subtypes of gastric adenocarcinoma (e.g., microsatellite instable subtype and P53 aberrant subtype) have been reported by different classification systems. However, the relation of these molecular subtypes to different clinicopathological features is still controversial.

Aim of the work: to utilize the immunohistochemical expression of DNA MMR proteins (MLH1 and MSH2) and p53 to detect microsatellite unstable and P53 aberrant molecular types in gastric adenocarcinoma. Moreover, to correlate immunohistochemically detected microsatellite unstable and P53 aberrant molecular types of gastric carcinoma with different clinicopathological characteristics.

Material & Methods: The immunohistochemical expression of MLH1, MSH2 and P53 proteins was evaluated in 70 cases of gastric adenocarcinoma. **Results:** Microsatellite status/Mismatch repair status showed a statistically significant relation with WHO classification, tumor differentiation, lymph node status and TNM staging. P53 aberrant type showed a statistically significant relation with tumor differentiation, depth of tumor invasion, lymph node status and TNM staging.

Conclusions & Recommendations: **Microsatellite instable GC** and P53 aberrant GC are two distinct molecular subtypes of gastric adenocarcinoma with distinct clinicopathological features and different prognostic outcome. **Microsatellite instable tumors** are associated with good prognostic parameters while, P53 aberrant tumors are associated with poor prognostic parameters. Both subtypes could be detected using immunohistochemistry and could represent potential targets for future therapeutic agents.

Key Words: MSI, MMR deficiency, MLH1, MSH2, P53, gastric adenocarcinoma

Introduction

Gastric cancer (GC) is among the five most frequent cancer [1] and the third most common causes for cancer-related fatalities worldwide [2]. Its prevalence is geographically variable and the majority of instances take place in developing nations and high-risk regions including Central and South America, Eastern Europe, and East Asian nations. [3].

GC is a varied and complicated disease caused by many interactions between genetic, environmental, and host variables. Despite attempts to enhance treatment methods over the last several decades, GC still has poor results [4].

Gastric cancer was traditionally classified based on morphology. The World Health Organization (WHO) categorized gastric adenocarcinoma into five major patterns in 2019: tubular, papillary, poorly cohesive, mucinous, and mixed adenocarcinomas, whereas the Lauren classification categorized gastric adenocarcinoma into (diffuse, intestinal, and mixed patterns) [5,6]. These morphology-based classification systems can't convey molecular heterogeneity nor guide the clinical practice for assessing the prognosis or anticipating the response of the therapy of GC patients. Therefore, identifying different subtypes of GC depending on genetic and molecular characteristics is essential for selection of targeted therapy [7].

The most thorough molecular categorization of GC was published in 2014 by The Cancer Genome Atlas (TCGA) Research Network. They suggested a molecular categorization that subcategorized GC into four subtypes: tumors with microsatellite instability (MSI), tumors with chromosomal instability (CIN), tumors positive for the Epstein-Barr virus (EBV), and tumors with genomic stability (GS) cancers [3]. The TP53 mutation rate was greatest in the CIN subtype [7], and P53 protein aberrations by immunohistochemistry (IHC) were subsequently utilized as a substitute for the CIN subtype [5].

Four molecular subtypes of GC were identified by The Asian Cancer Research Group (ACRG) in 2015: MSI, Microsatellite stable and epithelial to mesenchymal transition (MSS/EMT), MSS and TP53 active (MSS/TP53+), and MSS and TP53 inactive (MSS/TP53-) which showed the greatest rate of TP53 mutation [4].

Later on, other studies have reported more subtypes based on both TCGA and ACRG classifications [7] and the WHO (2019) stated that IHC of mismatch repair (MMR) proteins, P53

and epithelial-cadherin (E-cadherin) in combination with in situ hybridization (ISH) of EBV could allow molecular subtyping of GC in routine pathology [6].

MMR is the mechanism that recognizes and fixes base-base mismatches and insertions and deletions of DNA (deoxyribonucleic acid) that are created during the replication and recombination processes. This process is crucial for preserving genomic stability. Therefore, MMR faults are linked to genome-wide instability and a steady buildup of mutations, particularly in areas of the basic repeated DNA sequences known as microsatellites, which lead to MSI. [8]. Microsatellite instability is a resultant of defective MMR genes [mainly mutL homolog 1 (MLH1) and mutS homolog 2 (MSH2)] [9]. Therefore, identification of MSI can be accomplished by IHC of MMR proteins [1].

MMR-deficient cancers are presently seen as attractive targets for immunological checkpoint inhibition based on programmed cell death protein 1/programmed death-ligand 1 (PD1/PD-L1). This is because MMR-deficient cancers have PD-L1 gene amplification in a hypermutated phenotype with an increased tumor mutational burden[10].

The most frequently altered gene in instances of GC, found in 40% of patients, is the TP53 gene. The tumor suppressor protein P53 is essential for the halt of the cell cycle, metabolism, senescence, apoptosis, and DNA repair. Moreover, an important role of P53 is to maintain genomic stability so, it is called 'the guardian of the genome'[11].

P53 is the major executor of cell response to DNA damage and is thought to serve as a molecular hub for the communication between stressors (such as reactive oxygen species [ROS], nutritional deprivation, hypoxia, etc.) and cellular biological responses, P53 protein is defined as a stress response protein[12].

The present work aimed to utilize the immunohistochemical expression of DNA MMR proteins (MLH1 and MSH2) and p53 to detect microsatellite instability and P53 aberrant molecular types in gastric adenocarcinoma. Moreover, to correlate immunohistochemically detected microsatellite instability and P53 aberrant molecular types of gastric carcinoma with different clinicopathological characteristics.

Material and method

This retrospective investigation included 70 instances, primarily diagnosed as gastric adenocarcinoma in gastrectomy specimens (including 30 total gastrectomy specimens and 40 distal gastrectomy specimens), collected from the Pathology Department (Faculty of Medicine-Tanta University), Tanta Cancer Center and private laboratories during the period from June 2020 to January 2022. Prior to starting the study, the Tanta University Faculty of Medicine's Research Ethics Committee (REC) gave its approval. Approval code # 33827/5/20.

Clinicopathological data:

Clinicopathological data such as age, gender, tumor location, tumor size and distant metastasis were obtained from the cases' clinical sheets and pathology reports from the Pathology department (Tanta University), Tanta Cancer Center, and private laboratories files.

Histopathological evaluation:

Surgical samples were embedded in paraffin wax after being preserved in 10% neutral buffered formalin. Formalin-fixed paraffin-embedded block sections 3-5 μm thick were taken, and they were meticulously examined using standard H&E staining to confirm the histopathological diagnosis and assess various histopathological features like differentiation, lymph node status (N), depth of invasion (T), perineural invasion, and vascular invasion.

Cases of gastric adenocarcinoma were microscopically categorized as papillary, tubular, mucinous, poorly cohesive carcinoma (which includes signet ring cell carcinoma), and mixed adenocarcinomas, according to the World Health Organization (WHO) classification system, 5th edition, 2019 [6].

Tumor differentiation:

Tubular adenocarcinoma cases were subclassified based on histopathological grade from well to poorly differentiated by WHO criteria (2019). Well-differentiated tubular adenocarcinomas are predominantly made up of properly formed glands. In contrast, poorly differentiated tubular adenocarcinomas are made up of extremely irregular, ill formed glands and may have solid portions or individual cells, and moderately differentiated tumors show features that are "intermediate" between well and poorly differentiated tumors [6].

Regarding tumor differentiation, gastric adenocarcinoma cases were classified into differentiated and undifferentiated tumors. Differentiated tumors include moderately and well differentiated tubular and papillary adenocarcinomas, while undifferentiated tumors include poorly differentiated tubular adenocarcinomas, poorly cohesive carcinomas (including signet ring cell carcinomas), and others [13,14].

Staging of gastric adenocarcinoma cases:

The pathological staging of the examined gastric adenocarcinoma patients has been defined using the TNM staging strategy in accordance with the guidelines of the 8th edition of the AJCC, Cancer Staging Manual, 2017 [15,16]

Immunohistochemical staining:

Routine formalin fixed paraffin embedded (FFPE) sections, cut at 3 μm , were collected on positive charged slides. Slides were transferred to the Autostainer Link 48 instrument. Both high and low pH EnVision™ FLEX Target Retrieval Solutions (Dako, Burlington) were employed. Immunostaining was done with Dako and Spring bioscience primary antibodies. These were primarily Dako FLEX Ready to-Use format [MLH1 (*Kit no. E17810. Spring bioscience, Pleasanton, CA, USA*) and MSH2 (*Kit no. E17790. Spring bioscience, Pleasanton, CA, USA*), rabbit polyclonal antibodies, and P53 (*Kit no. M7001. Dako, Glostrup, Denmark*) mouse monoclonal antibody]. The Dako EnVision™ FLEX Detection system was then used, despite the absence of linker antibodies, in accordance with the standard protocol: 10 minutes for the peroxidase blocking reagent, 20–30 minutes for the primary antibodies, 20 minutes for the detection system, and 10 minutes for the chromogen (diaminobenzidine, or DAB). At the conclusion of the staining process, the slides were flooded with distilled water, a counter stain with Mayer's hematoxylin was applied for one minute, and the slides were finally washed in tap water. The slides were covered by using Canada balsam.[17]

Interpretation of MLH1 and MSH2 immunostaining

Tumor cells with brownish nuclear staining was considered positive regardless of cytoplasmic staining. When there was no trace of nuclear staining in any of the tumor cells, a tumor was deemed to be devoid of MLH1 or MSH2 expression (negative). As an internal positive control, peri-tumorous lymphocytes, stromal cells, and non-neoplastic epithelial cells were used [18]

Detection of microsatellite status (MS status)/Mismatch Repair status (MMR status):

Categorization of gastric carcinoma cases according to MLH1 and MSH2 expression is illustrated in table 1.

Table 1: Summary of the immunohistochemical interpretation of MLH1&MSH2

Category	Expression
MSI/MMR-deficient	MLH1 Negative or MSH2 Negative or Both MLH1 &MSH2 Negative
MSS/MMR-proficient	MLH1 Positive and MSH2 Positive

Mismatch repair status (MMR status) was determined by the immunohistochemical analysis of MLH1 and MSH2 expression [19]. Tumors negative for either MLH1 or MSH2 nuclear expression in all tumor cells were classified as MMR-deficient, whereas tumors with nuclear expression of both MLH1 and MSH2 were considered MMR-proficient[9]irrespective of the number or intensity of the stained tumor cell nuclei [20]

Microsatellite status (MS status): Gastric adenocarcinomas with brownish nuclear immunostaining of MLH1 protein (MLH1 positive) and MSH2 protein (MSH2 positive) in neoplastic cells were categorized as microsatellite stable tumors (MSS tumors/ tumors negative for MSI). Tumors showing complete loss of nuclear immunostaining for MLH1 protein (MLH1 negative) or MSH2 protein (MSH2 negative), in neoplastic cells, were categorized as MSI tumors[21].

Interpretation of P53 immunostaining:

Strong diffuse nuclear staining in more than 90 percent of tumor cells was the definition given for **aberrant expression of P53**, while the total lack of P53 expression in all tumor cells was the **alternative**; P53 expression was categorized as **wild type**[5]

Statistical analysis of the data:

Using software from the statistical package for the social sciences (SPSS), version 20.0, the gathered data was tabulated, arranged, and statistically evaluated. Calculations were done to determine the range, mean, and standard deviation of the quantitative data. The number of observations and the percentage of distribution were computed for the qualitative data. The significance of categorical variables was examined using the chi-square test so that comparisons could be made between the various groups. When more than 20% of the cells had an anticipated count of less than 5, Fisher's Exact correction and Monte Carlo correction were used as methods for correcting the chi-square test. Significance was adopted at p-value <0.05.

Results

This retrospective study was carried out on 70 cases primarily diagnosed as gastric adenocarcinomas. Clinicopathological variables of the studied cases are listed in **table 2**. The median age of the studied cases was 65 years ranging from 23-73 years with male predilection (38/70;54.3%). Most of the studied cases were located in antrum and pylorus (n=44;62.8%). The majority of cases measured more than 5 cm in diameter (50/70;71.4%). Tubular and poorly cohesive histological types were the most frequent in the studied cases (26/70;37.1% each). Forty-six cases (65.7%) showed undifferentiated morphology. Almost half of the cases (38/70; 54.3%) were T3 and 22 cases (31.4%) were N1 whereas stage II was detected in 32 cases (45.7%). Ten cases (14.3%) had distant metastasis. Lymphovascular invasion was detected in 34 cases (48.6%) and perineural invasion in 19 cases (27.1%).

Table 2: Classification of the studied gastric carcinoma cases according to different clinicopathological parameters

Clinicopathological variables	Number (%)
Age	
<60 years	29 (41.4)
≥60 years	41 (58.6)
Gender	
Male	38 (54.3)
Female	32 (45.7)
Tumor location	
Cardia and fundus	16 (22.9)
Body	10 (14.3)
Antrum and pylorus	44 (62.8)
Tumor size	
≤ 5 cm	20 (28.6)
> 5 cm	50 (71.4)
Histopathological types	
Tubular	26 (37.1)
Papillary	4 (5.7)
Poorly cohesive	26 (37.1)
Mucinous	10 (14.4)
Mixed	4 (5.7)
Tumor differentiation	
Differentiated	24 (34.3)
Undifferentiated	46 (65.7)
Depth of invasion	
T1	4 (5.7)
T2	20 (28.6)

T3	38 (54.3)
T4	8 (11.4)
Lymph node status	
N0	18 (25.7)
N1	22 (31.4)
N2	20 (28.6)
N3	10 (14.3)
Distant metastasis	
M0	60 (85.7)
M1	10 (14.3)
TNM stage	
Stage I	6 (8.6)
Stage II	32 (45.7)
Stage III	22 (31.4)
Stage IV	10 (14.3)
Vascular invasion	
Present	34 (48.6)
Absent	36 (51.4)
Perineural invasion	
Present	19 (27.1)
Absent	51 (72.9)
Total	70 (100)

Immunohistochemical results

MLH1 & MSH2 immunostaining results and classification of the studied cases according to microsatellite (MS) status/ mismatch repair (MMR) status

Out of 70 gastric adenocarcinoma cases, 55 cases (78.6%) were classified as microsatellite stable (MSS)/ MMR-proficient cases showing combined positive nuclear immunostaining of both MLH1 and MSH2 [Figure 1 and 2] while the remaining 15 cases (21.4%) were classified as tumors with microsatellite instability (MSI)/mismatch repair deficient (MMR-deficient) cases including 9 cases showing only MLH1 negativity (complete loss of MLH1 nuclear immunostaining) with positive MSH2 nuclear immunostaining [Figure 3 and 4] and 6 cases showing combined MLH1 and MSH2 negativity (complete loss of MLH1 & MSH2 nuclear immunostaining) [Figure 5 and 6] while none of the studied cases was only negative for MSH2 with positive MLH1 immunostaining. Distribution of the studied cases according to microsatellite (MS) status/mismatch repair (MMR) status is summarized in table 3.

Table 3: Distribution of the studied gastric carcinoma cases according to microsatellite (MS) status/mismatch repair (MMR) status:

Microsatellite (MS) status/mismatch repair (MMR) status		Number (%)	
MSI/MMR-deficient	MLH1 negative (with positive MSH2)	15 (21.4)	9 (12.8)
	Both MLH1&MSH2 negative		6 (8.6)
MSS/MMR-proficient	Both MLH1&MSH2 positive	55 (78.6)	
Total		70 (100)	

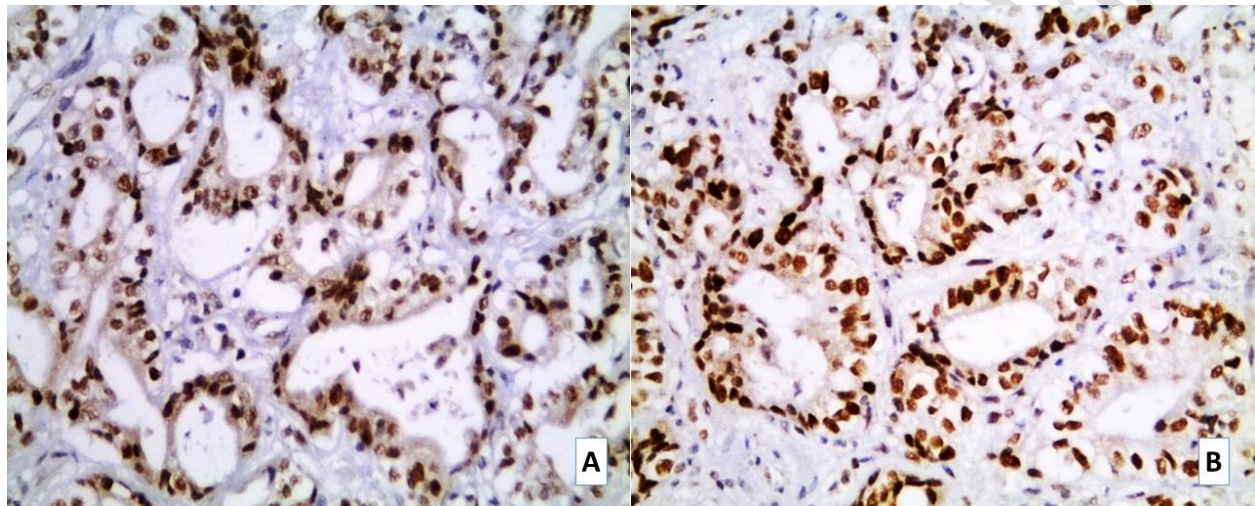


Figure 1: A case of moderately differentiated tubular adenocarcinoma classified as **microsatellite stable (MSS)/ MMR-proficient** showing combined nuclear positivity for **A: MLH1 B: MSH2** immunostaining (*streptavidin biotin x400*).

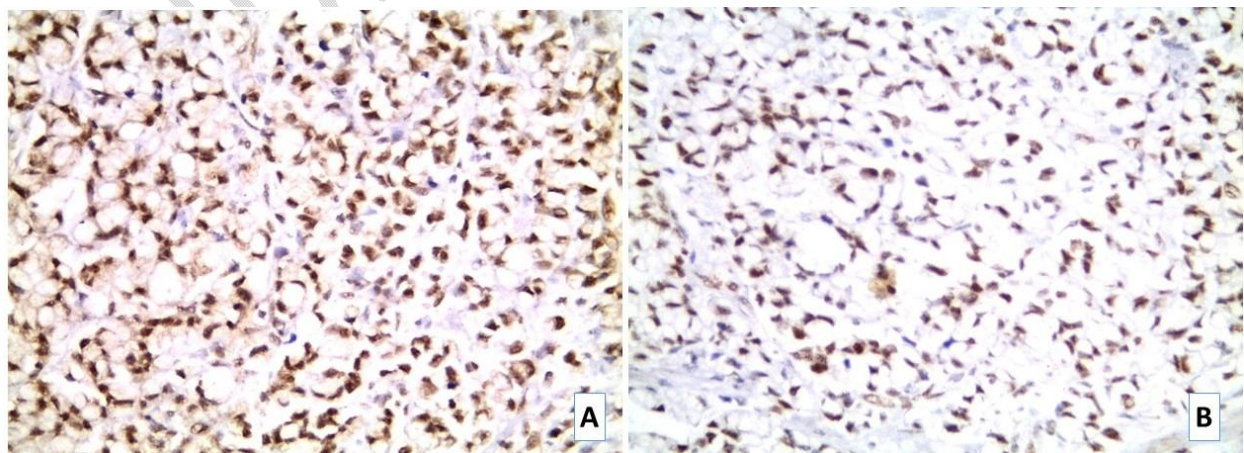


Figure 2: A case of poorly cohesive carcinoma, signet ring type adenocarcinoma classified as **microsatellite stable (MSS)/ MMR-proficient** showing combined nuclear positivity for **A: MLH1 B: MSH2** immunostaining (*streptavidin biotin x400*).

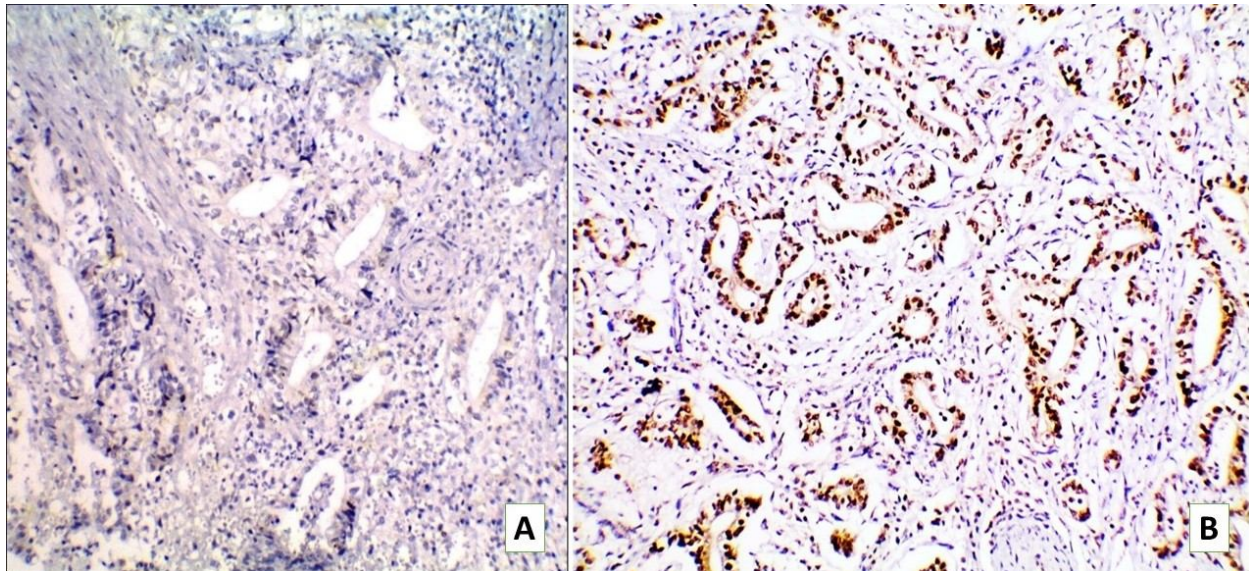


Figure 3: A case of well differentiated tubular adenocarcinoma (MSI/MMR-deficient case) showing **A: negative MLH1** and **B: positive MSH2** immunostaining (*streptavidin biotin x200*).

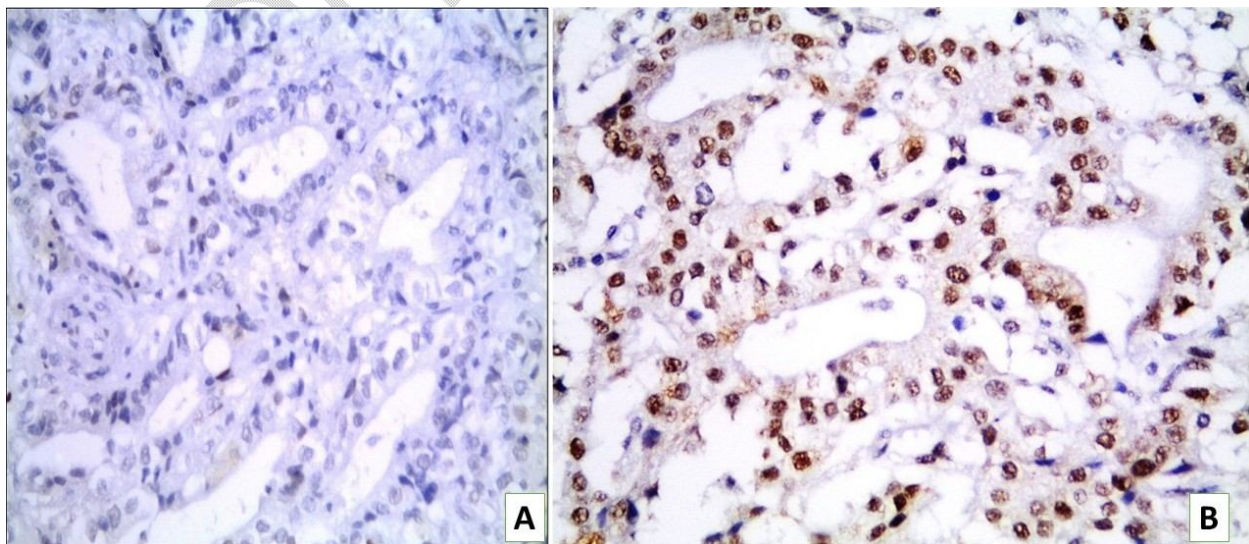


Figure 4: A case of moderately differentiated tubular adenocarcinoma (**MSI/MMR-deficient case**) showing **A:** negative MLH1 and **B:** positive MSH2 immunostaining (*streptavidin biotin x400*).

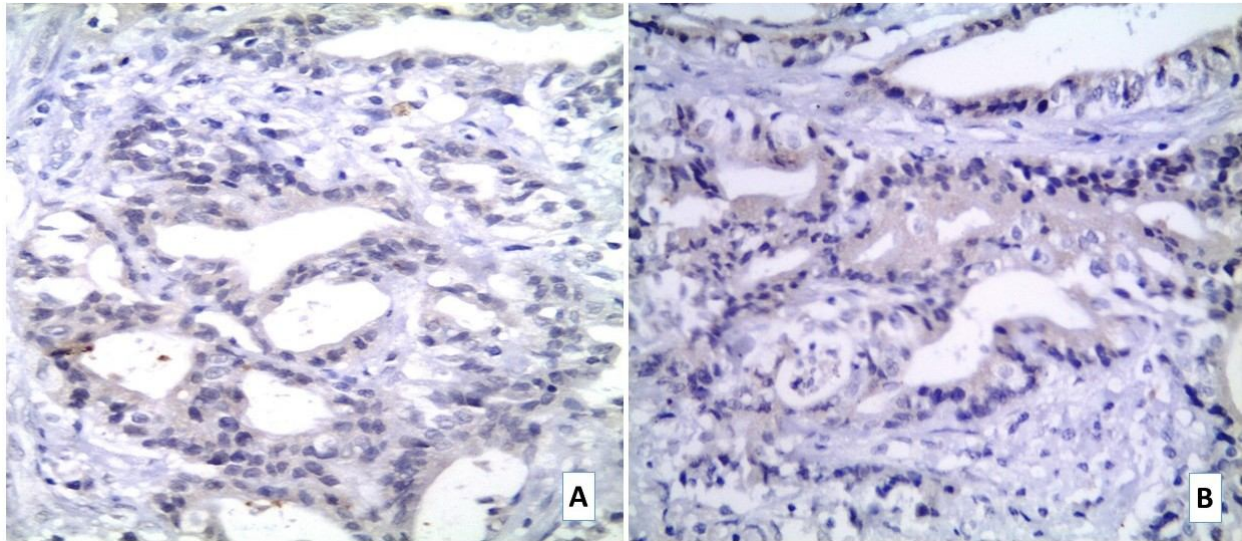


Figure 5: A case of moderately differentiated tubular adenocarcinoma classified as **MSI/MMR-deficient case** showing negative nuclear **A:** MLH1 and **B:** MSH2 immunostaining (*streptavidin biotin x400*).

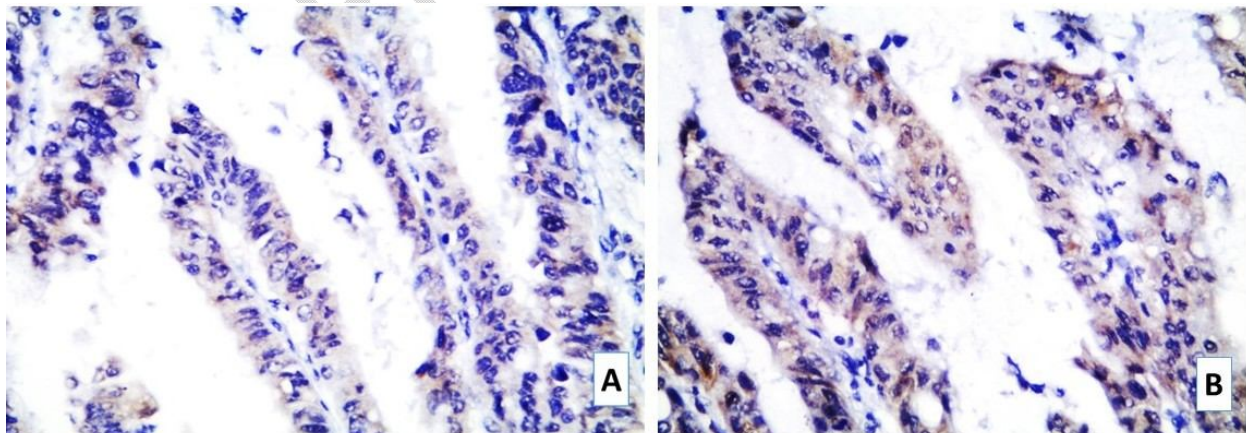


Figure 6: A case of papillary adenocarcinoma classified as **MSI/MMR-deficient case** showing negative nuclear **A:** MLH1 and **B:** MSH2 immunostaining (*streptavidin biotin x400*).

Relation between MS status/MMR status and clinicopathological characteristics of the studied cases of gastric carcinoma:

Relation between MS status/MMR status and the clinicopathological variables of the studied GC cases is listed in **table 4**. The relation between MS status/MMR status and histopathological types of the studied cases was statistically significant (with all papillary adenocarcinoma cases (100%) showing MMR deficiency/MSI) (p value=0.003). Regarding tumor differentiation, the relation between MS status/MMR status and tumor differentiation of gastric carcinoma cases was statistically significant with the majority of cases with MSI (9/15;60%) were of differentiated type (p value =0.018).

MS status/MMR status and lymph node status (N) was statistically significant with MSI/MMR-deficient cases associated with lower rate of lymph node metastasis (N0=7/15;47% and N1=6/15;40%) (Lower pN stage) compared to MSS/MMR-proficient cases (p value=0.045). The relation between MS status/MMR status and TNM staging of the studied cases was statistically significant with most cases with MSI were of stage I & II (13/15;87%) (Early stage) (P value=0.022).

Table 4: Relation between MS status/MMR status and the clinicopathological variables of the studied gastric carcinoma cases

Clinicopathologic variables	Total	MS status/MMR status		χ^2	P
		MSI/MMR-deficient N. (%)	MSS/MMR-proficient N.(%)		
Age					
<60 years	29	4 (13.8)	25 (86.2)	1.714	0.190
\geq 60 years	41	11 (26.8)	30 (73.2)		
Gender					
Male	38	6 (15.8)	32 (84.2)	1.570	0.210
Female	32	9 (28.1)	23 (71.9)		
Tumor location					
Cardia and fundus	16	3 (18.8)	13 (81.2)		

Body	10	2 (20.0)	8 (80.0)	0.124	1.000
Antrum and pylorus	44	10 (22.7)	34 (77.3)		
Tumor size					
≤ 5 cm	20	3 (15.0)	17 (85.0)		
>5 cm	50	12 (24.0)	38 (76.0)	0.687	0.528
Histopathological type					
Tubular	26	5 (19.2)	21 (80.8)		
Papillary	4	4 (100)	0 (0.0)		
Poorly cohesive	26	4 (15.4)	22 (84.6)		
Mucinous	10	2 (20)	8 (80.0)	16.40	0.003*
Mixed	4	0 (0.0)	4 (100.0)		
Tumor differentiation					
Differentiated	24	9 (37.5)	15 (62.5)		
Undifferentiated	46	6 (13.0)	40 (87.0)	5.603	0.018*
Depth of tumor invasion					
T1	4	1 (25)	3 (75.0)		
T2	20	6 (30)	14 (70.0)		
T3	38	8 (21.1)	30 (78.9)	3.088	0.371
T4	8	0 (0.0)	8 (100.0)		
Lymph node extension					
N0	18	7 (38.9)	11 (61.1)		
N1	22	6 (27.3)	16 (72.7)		
N2	20	2 (10.0)	18 (90.0)	7.984	0.045*
N3	10	0 (0.0)	10 (100.0)		
Tumor stage					
Stage I	6	3 (50)	3 (50.0)		
Stage II	32	10 (31.2)	22 (68.8)		
Stage III	22	2 (9.1)	20 (90.9)	9.459	0.022*
Stage IV	10	0 (0.0)	10 (100.0)		
Vascular invasion					
Absent	36	9 (25.0)	27 (75.0)		
Present	34	6 (17.6)	28 (82.4)	0.561	0.454

Perineural invasion					
Absent	51	10 (19.6)	41 (80.4)		
Present	19	5 (26.3)	14 (73.7)	0.370	0.531

*P value less than 0.5 was considered statistically significant

P53 immunohistochemical results and classification of the studied gastric adenocarcinoma cases according to P53 expression into aberrant and wild types

Representative images of p53 immunostaining are demonstrated in [Figure 7 and 8] Out of 70 gastric adenocarcinoma cases, 36 cases (51.4% of the studied cases) were classified as P53 aberrant type including 29 cases (41.4% of total cases) showing strong diffuse nuclear P53 immunostaining in more than 90% of tumor cells and 7 cases (10.0% of total cases) showing complete absence of P53 expression in all tumor cells. Thirty-four cases (48.6% of the studied cases) were classified as P53 wild type. Classification of the studied gastric carcinoma cases according to the P53 type is listed in **table 5**.

Table 5: Classification of the studied gastric carcinoma cases according to P53 expression:

P53 type		N. (%)	
P53 aberrant type	Strong diffuse immunostaining	36 (51.4)	29 (41.4)
	Negative immunostaining		7 (10.0)
P53 wild type		34 (48.6)	
Total		70 (100.0)	

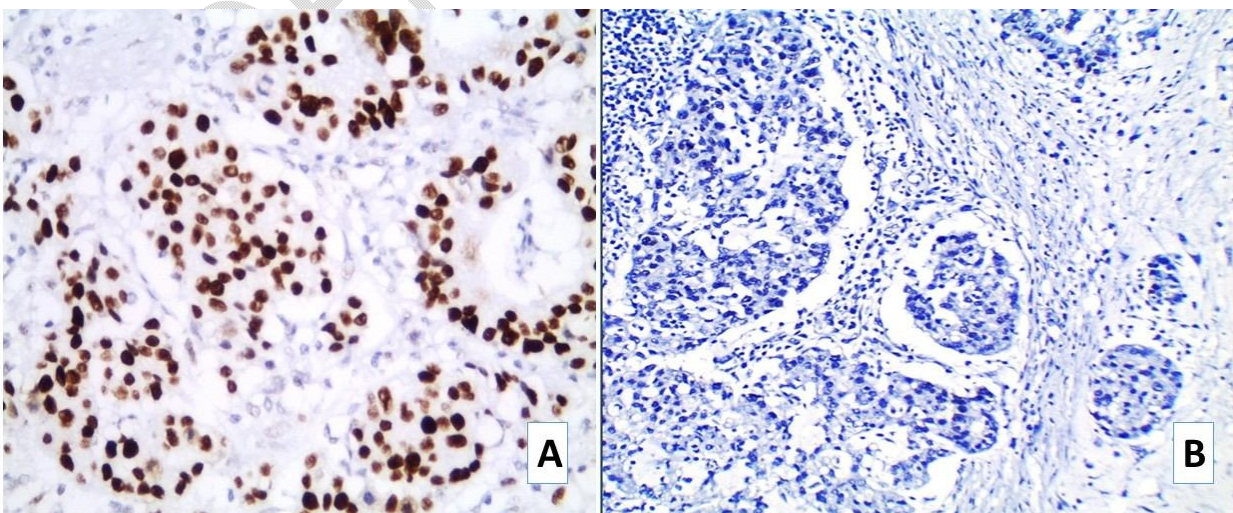


Figure 7: Representative images of p53 aberrant staining patterns:

A: case of poorly differentiated tubular adenocarcinoma, P53 aberrant type (*strong diffuse nuclear* P53 immunostaining) (*streptavidin biotin x400*).

B: A case of poorly differentiated tubular adenocarcinoma, P53 aberrant type (*negative nuclear* P53 immunostaining) (*streptavidin biotin x200*).

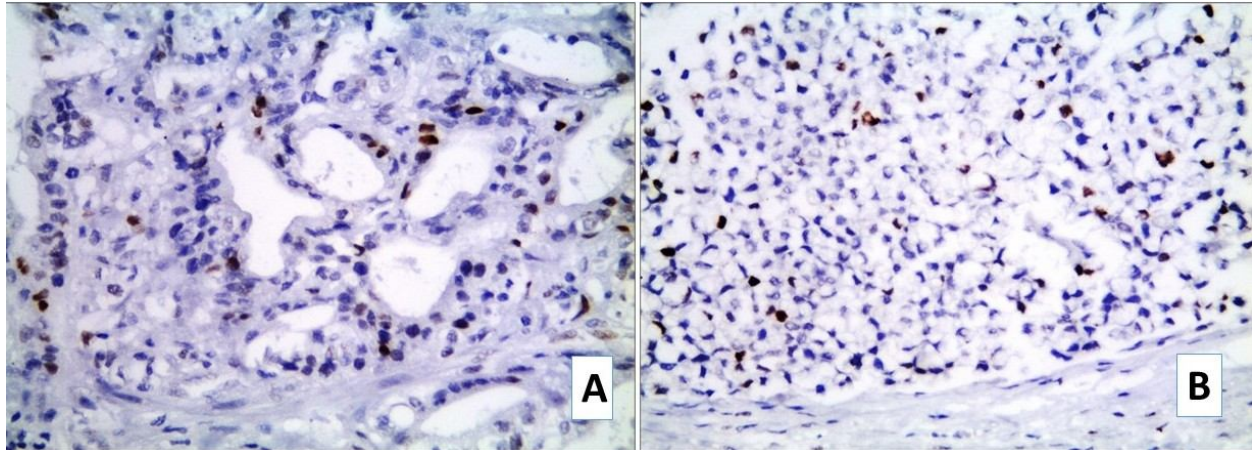


Figure 8: Representative images of p53 wild staining patterns:

A: A case of moderately differentiated tubular adenocarcinoma, P53 wild type (streptavidin biotin x400).

B: A case of poorly cohesive carcinoma (signet ring type), P53 wild type (streptavidin biotin x400).

Relation between P53 type and clinicopathological characteristics of the studied cases

Relation between P53 type and clinicopathological characteristics of the studied GC cases is summarized in **table 6**. The relation between P53 type and tumor differentiation of the studied cases was statistically significant with most undifferentiated cases (28/46;60.9%) showing p53 aberrant type (p value=0.029). The relation between P53 type and lymph node status (N) was statistically significant with the majority of cases in N2 (14/20;70%) and N3 (8/10;80%) categories (more frequent lymph node metastasis) of p53 aberrant compared to P53 wild type (p value=0.006). A statistically significant relation between P53 type and TNM staging of the studied cases was found (p value<0.001), with most of the stage III (18/22;81.8%) and stage IV (8/10;80%) (Advanced stage) cases classified as p53 aberrant type.

Table 6: Relation between P53 type and clinicopathological characteristics of the studied gastric carcinoma cases

Clinicopathological variables	N.	P53 type		χ^2	P value
		P53 aberrant type	P53 wild type		
		N.(%)	N.(%)		
Age					
<60 years	29	17 (58.6)	12 (41.4)	1.025	0.311
≥60 years	41	19 (46.3)	22 (53.7)		
Gender					
Male	38	23 (60.5)	15 (39.5)	2.754	0.097
Female	32	13 (40.6)	19 (59.4)		
Tumor location					
Cardia and fundus	16	12 (75.0)	4 (25.0)	5.802	0.055
Body	10	6 (60.0)	4 (40.0)		
Antrum and pylorus	44	18 (40.9)	26 (59.1)		
Tumor size					
≤ 5 cm	20	10 (50.0)	10 (50.0)	0.023	0.880
> 5 cm	50	26 (52.0)	24 (48.0)		
Histopathological type					
Tubular	26	12 (46.2)	14 (53.8)	4.654	0.372
Papillary	4	2 (50.0)	2 (50.0)		
Poorly cohesive	26	12 (46.2)	14 (53.8)		
Mucinous	10	6 (60.0)	4 (40.0)		
Mixed	4	4 (100.0)	0 (0.0)		

Tumor differentiation					
Differentiated	24	8 (33.3)	16 (66.7)	4.78	0.029*
Undifferentiated	46	28 (60.9)	18 (39.1)		
Depth of invasion					
T1	4	2 (50.0)	2 (50.0)	11.257	0.008*
T2	20	6 (30.0)	14 (70.0)		
T3	38	20 (52.6)	18 (47.4)		
T4	8	8 (100.0)	0 (0.0)		
Lymph node status					
N0	18	4 (22.2)	14 (77.8)	12.490	0.006*
N1	22	10 (45.5)	12 (54.5)		
N2	20	14 (70.0)	6 (30.0)		
N3	10	8 (80.0)	2 (20.0)		
TNM stage					
Stage I	6	2 (33.3)	4 (66.7)	21.136	<0.001*
Stage II	32	8 (25.0)	24 (75.0)		
Stage III	22	18 (81.8)	4 (18.2)		
Stage IV	10	8 (80.0)	2 (20.0)		
Vascular invasion					
Absent	36	16 (44.4)	20 (55.6)	1.447	0.229
Present	34	20 (58.8)	14 (41.2)		
Perineural invasion					
Absent	51	23 (45.1)	28 (54.9)	3.014	0.083
Present	19	13 (68.4)	6 (31.6)		
MS status/ MMR status					
MSI/MMR-deficient	15	5 (33.3)	10 (66.7)	2.502	0.114
MSS/MMR-proficient	55	31 (56.4)	24 (43.6)		

***P value less than 0.5 was considered statistically significant**

Relation between MS status/MMR status and P53 type of the studied cases

Most MSI/MMR-deficient cases (10/15; 66.7%) were P53 wild type. However, the relation between MS status/MMR status and P53 type of the studied cases didn't reach a statistically significant value.

Discussion

GC is the world's fifth most frequent cancer, behind lung, breast, colorectal, and prostate cancer. In terms of total mortality, it is the third greatest cause of cancer deaths globally, after only lung and colorectal cancer[22]. In Egypt, GC is the 12th most prevalent cancer in both sexes, accounting for 1.6% of all cancers, and it is the 12th greatest cause of cancer death, accounting for 2.2% of all cancer deaths[23].

The molecular classification of GC was a big step forward since it represents tumor biology and may be linked to specific clinicopathological facts[24]. It is deemed critical for GC diagnosis [25] and selecting targeted treatments[11]. The most thorough molecular categorization of GC was published in 2014 by TCGA Research Network. They suggested a molecular categorization that subcategorized GC into four subtypes: tumors with MSI, CIN, tumors positive for the EBV, and tumors with GS cancers [3].

Four molecular subtypes of GC were identified by ACRG in 2015: MSI, MSS/EMT, MSS/TP53+, MSS/TP53- which showed the greatest rate of TP53 mutation[4]. The TCGA and ACRG classification systems employed expensive and advanced high throughput technology. However, this sophisticated methodological approach may not be adopted into normal surgical specimen processing in the near future [26].

It has recently been proven that molecular subtyping of GC is possible using immunohistochemistry (IHC) and in situ hybridization (ISH) [27]. Setia et al., [28] offered a simplified and less expensive algorithm for GC molecular subtyping. They offered procedures often used in diagnostic practice, such as ISH and IHC, and found five GC groups that match to TCGA and ACRG molecular subgroups: EBV+, mismatch repair-deficient, aberrant E-cadherin expression, normal p53 expression, and aberrant p53 expression [28]. The WHO (2019)

recommended that in conventional pathology, IHC of mismatch repair proteins (MLH1, etc.), p53, and E-cadherin coupled with ISH for EBV-encoded small RNA would provide molecular GC subtyping [6].

MMR is a highly conserved DNA repair mechanism that recognizes and repairs single-base mismatches that elude polymerase proof-reading activity [29]. Because of its active involvement in preserving DNA integrity, this mechanism is critical for cell homeostasis [30]. Tumors with MMR dysfunctions are more likely to be hypermutators [31].

Although PCR-based molecular testing has been considered a standard diagnostic method for detection of microsatellite instability, IHC is simpler, quick, and widely available than molecular testing. IHC showed a high concordance rate compared with PCR-based assays in detection of MSI. IHC demonstrated sensitivity of 91.1% and specificity of 98.5% compared to PCR-based analysis in the identification of MSI phenotype in gastric cancer [32]. Additionally, Kim et al. [33], stated that IHC achieved a similar level of specificity and sensitivity as PCR-based molecular testing of MSI.

The rationale for using IHC of MMR proteins for assessment of MSI was mentioned in previous reports as follows: IHC of MMR proteins has high sensitivity and specificity in addition to positive and negative predictive values for the mismatch repair system deficiency [28,34]. Furthermore, the main mechanism of microsatellite instability in gastric cancer is promoter hypermethylation of MLH1, less commonly, mutations in MLH1 and MSH2 [35]. Moreover, MLH1 IHC detection has showed highly concordant results compared to PCR assessment [36]. As a result, IHC may be used to test for MMR deficiency in GCs in a straightforward and reliable manner.

Given the reliability, cost effectiveness of IHC, availability of MMR antibodies in pathology laboratories, and that most cases show straight forward interpretation, this method (IHC) is widely considered as a first-line diagnostic test [37–39].

In agreement with Bae et al. [32], Kim et al. [33], Inada et al., (2015) & Giampieri et al. [9] The present study identified MMR-defective tumors based on the lack of MLH1 or MSH2 expression, regardless of the expression of MSH6 or PMS2 in the tumor. Although MSH6 and PMS2 expression are well known to be important factors in the definition of MMR activity in other tumors (such as colon cancer), it seems in GC that these two genes are less relevant, with MLH1-defective status being the alteration most frequently found in MMR-defective tumors.

In the current study, 21.4% of the studied gastric adenocarcinoma cases were classified as tumors with microsatellite instability (MSI)/mismatch repair deficient (MMR-deficient) cases while 78.6% of cases were classified as microsatellite stable (MSS)/ MMR-proficient cases. This frequency was consistent with previous reports [40–42] but higher than [43–45] and lower than other studies [46,47].

The discrepancies between the different studies may be explained by the variability in population size and population characteristics, variability of antibodies and differences of interpretation of expression profiling (variation of the scoring system or the cutoff levels used).

Other cause of discrepancy is using whole surgical section versus tissue microarray. In agreement with Bosch et al. [5], and Di Pinto et al. [43], the current investigation used ICH on the whole section of surgical GC specimens, while most previous studies used tissue microarrays (TMAs), which have been proven not to be accurate representations of intratumor heterogeneous protein expression [27,28,48,49]. As a result, assessing the whole tumor region is critical for a more precise assessment of the MMR status.

The present study aimed to correlate the MS status/MMR status of the GC studied cases with clinicopathologic variables. As regard to the histopathological type of gastric carcinoma, the majority of previous studies were not concerned with WHO histopathological subtype, but rather with Lauren's classification and Lauren's intestinal-type histology predominated in **microsatellite unstable gastric carcinomas** [1,5,14,27,28,32,41,49,50].

In the current study, we studied the relationship between WHO histopathological subtypes of gastric carcinomas and microsatellite status to examine histological diversity associated with MSI. The relation between MS status/MMR status and WHO histopathologic types of the studied gastric adenocarcinoma cases was statistically significant with all papillary adenocarcinoma cases showing MMR deficiency (MSI). Similarly, Sugimoto et al. [46], reported that the relation between MSS and histological type of tumor (Papillary versus Non papillary) was statistically significant with most papillary adenocarcinoma cases showing MSI-high phenotype and Arai et al [51]., stated that the proportion of MSI is significantly correlated with certain histological subtype (such as papillary adenocarcinoma and solid-type poorly differentiated adenocarcinoma). This finding was inconsistent with previous reports [47,52]. Additionally, Setia et al. reported that MSI-gastric cancer subgroup included multiple WHO

patterns but carcinoma with lymphoid stroma was significantly associated with microsatellite instability compared to other molecular subtypes[53].

It has been hypothesized that papillary adenocarcinoma is a separate morphological form with a molecular pathway of MSI since it has been discovered that **microsatellite-unstable** papillary carcinoma exhibits preferential hypermethylation of the hMLH1 promoter, with an absence of hMLH1 expression, and frequent mutations in genes such as BAX, MSH3, and MSH6. In early GC, the papillary type was the one that revealed centromere numerical abnormality[51].

In the present study, a statistically significant relation between MS status/MMR status and tumor differentiation was found in the studied cases with the majority of cases with MSI were of differentiated type which was consistent with Bae et al.[32], Park et al.[14],and Vermaet al[47] who reported that MMR deficiency (MSI) was significantly associated with more differentiated histology (compared to MMR-P/MSS cases) and Ahn et al. [49] who reported significant association of high-MSI tumors with better differentiated histology (compared to other molecular subtypes).

On the contrary, previous reports showed no significant association between MMR and histological differentiation/tumor grade[45,50,54] and Bösch et al. reported a significant association of different molecular subtypes with tumor grade with both MMR/MSI and aberrant p53 subtypes were more associated with increased G3 probability (poorly differentiated)[5].

Arai et al. [51], showed no significant difference in the frequency of MSI between the differentiated and the undifferentiated type of GC. However, they reported that poorly differentiated carcinomas were significantly less common than differentiated carcinomas in microsatellite-unstable cancer at the early stage, whereas no significant difference found at the advanced stage. They proposed that gastric carcinoma with MSI develops principally as a differentiated-type tumor then progresses to a more poorly differentiated tumor (at the advanced stage).

The present study found an insignificant relation between MS status/MMR status and depth of tumor invasion (T) which was consistent with previous reports [47,50,54,55]. On the contrary, Dislich et al.[1]& Ito et al. [45] reported that d-MMR/MSI-H cases were significantly associated with shallower tumor depth (lower pT-category) compared to MMR-P/MSS cases.

MMR deficient cases in the present study were significantly associated with lower rate of lymph node metastasis compared to MMR proficient cases. This finding was in line with previous reports [1,45,50,55]. Besides, Setia et al. [28], reported that MSI subtype was significantly associated with decreased rate of lymph node metastasis (lower pN stage) compared to other molecular subtypes. This finding also reflects lesser biologic aggressiveness and a trend towards longer survival in this group. Additionally, Arai et al.[51], reported a statistically significant relation between MSI and lower rate of lymph node metastasis at advanced stage but no significant relation was detected in early stage compared to MSS cases. On the contrary, MMR deficiency/MSI was not significantly associated with lymph node metastasis in other studies[18, 47,52,54]

In the present study the relation between MS status/MMR status and TNM staging of the studied gastric carcinoma cases was statistically significant with the majority of cases with d-MMR/MSI were of stage I & II (early stage) which was consistent with previous studies [32,41,45,50,52,56]. Conversely, This finding was inconsistent with other studies [18,43,55].

The present study revealed an insignificant relation between MS status/MMR status and vascular invasion in the studied gastric adenocarcinoma cases. This was in line with previous studies [18,47,54]. On the contrary, Park et al.[14] reported that MSI-H was significantly related to presence of lymphovascular invasion (LVI) compared to MSS cases and Ito et al.[45] reported that d-MMR cases were significantly associated with more frequent presence of venous invasion (but not significantly associated with lymphatic invasion) while Tsai et al. stated that d-MMR cases were significantly associated with less frequent vascular invasion (but not significantly associated with lymphatic invasion) compared to MMR-P cases[50].

The relation between MS status/MMR status and perineural invasion in the studied cases was statistically insignificant in the present study. This finding was consistent with[55,57]and inconsistent with previous studies[50,54]

One weakness of our research is the absence of long-term follow-up, which results in a lack of overall survival statistics. However, the comprehensive pathological variables that are accessible show a prognostic relationship between patients with MMR-deficient tumors and a better disease course. This is indicated by a lower frequency of lymph node metastases, a lower tumor stage, and better differentiation, all of which represent a lesser biologic aggressiveness. These observations are consistent with the previously published studies who reported that

dMMR/MSI carcinomas have the best survival and the best prognosis between all GC molecular subtypes[44,56,58,59]. Additionally, Bae et al.[32]and Zhang et al [60] also reported that patients with dMMR phenotype had improved survival and better prognosis compared to those with pMMR status.

The considerable T cell infiltration in these tumors may explain the better survival of individuals with MSI gastric cancer. Indeed, frame-shift mutations that produce aberrant peptides that may be delivered to cytotoxic T cells are common in MSI malignancies [59,61].

Ramos et al. [56]reported that MSI GC was the subtype with highest survival rates. It's still unclear why tumors associated with MSI commonly have better prognosis. The lymphocytic peritumoral infiltration in these types of tumors, in response to peptides produced by MSI tumors, is thought to contribute considerably to the anti-tumor response by triggering tumor cell death through cytokine activation[62]. The lower LN involvement, which is a hallmark of this subtype in addition to other characteristics of these tumors, can also be related to their favorable prognosis. Even in situations when the pN status is positive, metastases often only impact a limited number of LNs[63]. Moreover, Martinez-Ciarpaglini et al.[18] stated that in multivariate analysis, MSI was substantially linked to a lower risk of death, regardless of other variables such as clinical stage or nodal metastases. Due to the prognostic significance of MSI testing in GC patients, we advise that it be used in clinical practice. Furthermore, MSI may provide useful information to avoid neoadjuvant chemotherapy in cases with limited locoregional disease or to consider immunotherapy in relapsing or advanced stage cases [57].

On the contrary, Arai et al. [51] demonstrated that there was no significant difference in prognosis between MSS and MSI gastric cancers and Kim et al. [64]reported that tumors with MSI appear to be linked to worse prognosis.

The transcription factor p53 is a key tumor suppressor protein in humans, which is mutated in more than half of all human malignancies along their progression. Mutant p53 is regarded as a promising and unique drug target in the field of cancer therapy because of its frequent involvement in cancer progression[65,66]

Normal p53 [wild type] have short half-life that cannot be stained [67]. The majority of TP53 mutations are missense mutations, and IHC results demonstrate that p53 is overexpressed as a result of the buildup of non-functional proteins [42]. Proteins with a longer half-life are produced by TP53 mutations, and since they build up in the nucleus, they are overexpressed. On

the other hand, a truncated protein can result from some missense or point mutations that is not stable enough to cause any noticeable nuclear accumulation. Therefore, the absence or increased p53 nuclear expression can be used to identify its aberrant expression [56,68]

Additionally, Grosser et al.[69] performed mutational analysis of the TP53 gene by next-generation sequencing (NGS) and a concordant result with aberrant p53 expression (including overexpression and complete loss) by IHC analysis were demonstrated in (90%) cases. Hwang et al.[70] stated that IHC results showed that TP53 missense mutations were strongly correlated with strong p53 expression, other kinds of mutations were negatively correlated with high p53 expression, and wild-type TP53 was correlated with weak p53 expression (p value 0.001). Each category's sensitivity and specificity were respectively 90.9%, 79.0%, and 80.9% and 95.4%, 88.1%, and 92.3%. Therefore, p53 protein IHC can be used as a simple surrogate marker of TP53 mutations[69].

In a study that combined an IHC labeling pattern associated with TP53 mutations (0% and 60-100% positive cells) and a nucleotide sequence analysis of p53 in ovarian cancer, the researchers found that combining the two patterns correctly identified a mutation in 94% of instances (P 0.001). They came to the conclusion that IHC is a valid approach for determining if ovarian cancer had the TP53 mutation[71]. A three-tiered scoring system, comprising overexpression, total absence, and a normal or wild-type pattern in ovarian cancer, was suggested by Kobel et al. [72] for the interpretation of P53 IHC. The scoring method showed a strong correlation with the TP53 mutation status, showing overexpression in the presence of nonsynonymous mutations, total absence in the presence of stop gain, frameshift, and splicing mutations, and a typical pattern in the presence of the wild-type TP53 gene.

As regard gastric adenocarcinoma, it was recently demonstrated that immunohistochemical (IHC) staining (which is widely available techniques in routine diagnostic practice) could be used as a surrogate for TP53 gene mutational analysis to approximate the results in a simple and cost-effective manner[27,49,70]. As the CIN subtype of TCGA molecular classification (2014) showed the highest frequency of P53 mutations, therefore P53 protein aberrations by immunohistochemistry can be used as a surrogate for CIN subtype [5]

Ando et al.[67], stated that; only positive or negative p53 staining had no effect in determining the mutation status of TP53 gene in gastric cancer. They reported that; in positive p53 tumors (showing 10% or more nuclei stained with p53), two types of staining could be

distinguished; aberrant type (staining of 70% or more nuclei) and scattered type (20–50% of nuclei stained) and they observed a significant relation between p53 staining pattern and TP53 gene mutations. It's interesting to note that all cancers with the dispersed tumor type had wild-type TP53 genes, and all tumors with missense mutant TP53 genes exhibited abnormal p53 staining[67].

Aberrant expression of p53 by immunohistochemistry has been assessed differently in GC: some studies rated tumor cells p53 overexpression using different cutoffs [36][48], whereas others included the loss of p53 immunostaining as aberrant expression [28][49]. Daun et al. [73] combined p53 IHC, including loss and overexpression, and TP53 gene sequencing by NGS technique for detection of P53 aberrant cases.

Various studies have used various cutoffs to estimate p53 overexpression in tumor cells when assessing p53 aberrant expression in GC [36][48], while other research have considered the loss of p53 immunostaining as an aberrant expression [28][49]. For the purpose of identifying P53 aberrant expression, Daun et al. [73] coupled p53 IHC, including loss and overexpression, with TP53 gene sequencing using the NGS method.

Grosser et al.[69]& Kim et al. [74] defined tumors with aberrant p53 expression as tumors showing complete absence or diffuse and strong p53 nuclear positivity. In the current study we interpreted strong diffuse nuclear staining in > 90% of tumor cells or complete absence of p53 expression in all tumor cells as aberrant expression of p53 otherwise; p53 expression was categorized as wild type as reported by Bosch et al. [5].

In the present study, 51.4% of the studied gastric carcinoma cases were classified as P53 aberrant type while 48.6% were classified as P53 wild type.

The relation between P53 type and tumor differentiation of the studied GC cases was statistically significant in the present study with the majority of cases classified as p53 aberrant type showing undifferentiated morphology which was consistent with Bösch et al.[5]who reported significant association of different molecular subtypes with tumor grade (with both dMMR/MSI and aberrant p53 subtypes were more associated with an increased G3 probability/poorly differentiated).

On the contrary, Birkman et al.[27] reported no significant association between P53 expression and histological grade among intestinal type tumors. Kim et al.[75] reported that P53 overexpression/null tumors (corresponding to P53 aberrant subtype) were significantly

associated with more differentiated histology compared to p53 weak group and Ramos et al.[56] reported a statistically significant association between different molecular subtypes and histological grade differentiation with the p53 aberrant tumors were more frequently well or moderate differentiated tumors. Additionally, Ando et al.[67] reported that P53 mutation (detected by TP53 gene mutation analysis) showed a statistically significant association with tumor differentiation with TP53 gene mutation found more frequent in differentiated than in undifferentiated type.

The present study revealed that the relation between P53 type and depth of tumor invasion (T) was statistically significant with the majority of cases classified as p53 aberrant type showing T3&T4 categories (deeper tumor invasion). This was inconsistent with previous studies [5,27,56,73]

The relation between P53 type and lymph node status (N) was statistically significant in the present study with the majority of gastric carcinoma cases classified as p53 aberrant type showed N2&N3 categories (more frequent lymph node metastasis) which was consistent with Setia et al.[28] who reported that GC with aberrant p53 expression were significantly associated with higher lymph node stage compared to other molecular subtypes and Grosser et al.[69], who reported that aberrant p53 expression was associated with a positive lymph node status. On the other hand, the relation between P53 expression and lymph node extension was statistically insignificant in previous studies [5,56,67].

The present study revealed a statistically significant relation between P53 type and TNM staging of the studied gastric carcinoma cases was with the majority of cases classified as p53 aberrant type showing stage III & stage IV (advanced stage) which was consistent with Hwang et al. [70] who reported that; the TNM stage at the first diagnosis showed a significant correlation with p53 expression status with most cases showing strong P53 expression were of stage III. This finding was inconsistent with others[27,44,56,67,75]

A limitation of the present study is the absence of prolonged follow-up and absent overall survival data. However, the available pathological parameters show a prognostic association between patients with P53 aberrant tumors and poor prognostic parameters indicating a more aggressive disease course, with deeper depth of tumor invasion, more frequent lymph node metastases, higher tumor stage and more undifferentiated histology which reflects more biologic aggressiveness of P53 aberrant tumors compared to P53 wild type tumors.

Similarly, Grosser et al.[69] reported that aberrant p53 expression was significantly associated with worse overall survival in the total resected tumor cohort. The ACRG [41]Bösch et al.[5] Pinto et al.[44]&Nshizirungu et al.[59] reported that P53 aberrant carcinomas have intermediate prognosis and survival compared to other molecular subtypes of GC. Ahn et al. [49]& Kim et al.[64] reported that MSI tumors had the best prognosis, followed by the EBV tumors; cases with normal p53 expression, aberrant p53 expression, and EMT/GS tumor showing the worst prognosis. Gurzu et al.[76] reported that TP53 wild-type cases had a longer survival, compared with P53 mutant GCs but didn't reach a statistically significant value. On the contrary, no significant survival difference was noted in GC with aberrant p53 expression compared to the remaining adenocarcinomas in other studies [27,28]

The present study revealed that most MSI/MMR-deficient cases (66.7%) were p53 wild type but the relation between MS status/MMR status and P53 type of the studied cases didn't reach a statistically significant value. Similarly, Gonzalez et al.[48] stated that TP53 mutation can occur in cancers associated with MSI, though there is no statistically significant link of MSI and P53 aberrant type cases.

On the other hand, Birkman et al.[27] reported a significant association between P53 expression and microsatellite status with TP53 aberrant tumors were more frequently MSS than MSI. Hwang et al.[70] reported that p53 expression showed a significant association with MSI status with all p53 aberrant cases (strong P53 expression and negative P53 expression) were of the MSS/MSI-L type. Additionally, Grosser et al.[69] also reported a strong association of aberrant p53 with MSI-L.

Setia et al.[28]stated that MSI GC have been identified as a distinct group based on clinicopathologic and molecular characteristics. Yet, this subset may have aberrant p53 expression along with TP53 anomalies (most frequently because of loss of heterozygosity).

Conclusions:MSI phenotype GC and P53 aberrant GC are distinct molecular subtypes of gastric adenocarcinoma with distinct clinicopathological features and different prognostic outcomes. MSI/MMR-deficient gastric tumors are associated with good prognostic parameters with a lower frequency of lymph node metastases, lower tumor stage and better differentiation which reflects lesser biologic aggressiveness compared to MSS/MMR-proficient tumors. P53 aberrant gastric tumors are associated with poor prognostic parameters with deeper depth of

tumor invasion, more frequent lymph node metastases, higher tumor stage and more undifferentiated histology which reflects more biologic aggressiveness of P53 aberrant tumors compared to P53 wild type tumors.

Recommendations: Further studies should be carried out to investigate the patients' survival in MSI GC and P53 aberrant GC. Large scale studies including large number of GC patients could be used to validate the findings of the present study. Different molecular subtypes of gastric adenocarcinoma with distinct molecular characteristics can represent potential targets for future therapeutic agents and further understanding of gastric cancer biology. Further molecular studies should be carried out in the future seeking more molecular properties that could be applied using immunohistochemical staining for better identification of different molecular subtypes of GC.

References

1. Dislich B, Blaser N, Berger MD, Gloor B, Langer R. Preservation of Epstein–Barr virus status and mismatch repair protein status along the metastatic course of gastric cancer. *Histopathology*. 2020 Apr;76(5):740–7.
2. Song WM, Lin X, Liao X, Hu D, Lin J, Sarpel U, et al. Multiscale network analysis reveals molecular mechanisms and key regulators of the tumor microenvironment in gastric cancer. *Int J Cancer*. 2020 Mar;146(5):1268–80.
3. Martinson HA, Mallari D, Richter C, Wu TT, Tiesinga J, Alberts SR, et al. Molecular classification of gastric cancer among Alaska native people. *Cancers (Basel)*. 2020 Jan;12(1).
4. Serra O, Galán M, Ginesta MM, Calvo M, Sala N, Salazar R. Comparison and applicability of molecular classifications for gastric cancer. *Cancer Treat Rev*. 2019 Jul;77:29–34.
5. Bösch F, Todorova R, Link H, Westphalen CB, Boeck S, Heinemann V, et al. Molecular subtyping of gastric cancer with respect to the growth pattern of lymph-node metastases. *J Cancer Res Clin Oncol*. 2019 Nov;145(11):2689–97.
6. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology*. 2020 Jan;76(2):182–8.
7. Wang Q, Liu G, Hu C. Molecular Classification of Gastric Adenocarcinoma. *Gastroenterol Res*. 2019 Dec;12(6):275–82.
8. Lorenzi M, Amonkar M, Zhang J, Mehta S, Liaw KL. Epidemiology of Microsatellite Instability High (MSI-H) and Deficient Mismatch Repair (dMMR) in Solid Tumors: A

- Structured Literature Review. Roviello G, editor. *J Oncol* [Internet]. 2020;2020:1807929. Available from: <https://doi.org/10.1155/2020/1807929>
9. Giampieri R, Maccaroni E, Mandolesi A, Del Prete M, Andrikou K, Faloppi L, et al. Mismatch repair deficiency may affect clinical outcome through immune response activation in metastatic gastric cancer patients receiving first-line chemotherapy. *Gastric Cancer*. 2017 Jan;20(1):156–63.
 10. Chénard-Poirier M, Smyth EC. Immune Checkpoint Inhibitors in the Treatment of Gastroesophageal Cancer. *Drugs*. 2019 Jan;79(1):1–10.
 11. Cisło M, Filip AA, Offerhaus GJA, Ciseł B, Rawicz-Pruszyński K, Skierucha M, et al. Distinct molecular subtypes of gastric cancer: From Laurén to molecular pathology. *Oncotarget*. 2018 Apr;9(27):19427–42.
 12. Amelio I, Melino G. Context is everything: extrinsic signalling and gain-of-function p53 mutants. *Cell Death Discov* [Internet]. 2020;6(1):16. Available from: <https://doi.org/10.1038/s41420-020-0251-x>
 13. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med*. 2018 Sep;24(9):1449–58.
 14. Park JH, Kim EK, Kim YH, Kim JH, Bae YS, Lee YC, et al. Epstein–Barr virus positivity, not mismatch repair-deficiency, is a favorable risk factor for lymph node metastasis in submucosa-invasive early gastric cancer. *Gastric Cancer*. 2016 Oct;19(4):1041–51.
 15. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more “personalized” approach to cancer staging. Vol. 67, CA: A Cancer Journal for Clinicians. United States; 2017. p. 93–9.
 16. Si Chun Ming. Tumors of the Esophagus and Stomach. In: Fletcher, editor. *AmRegPathol*. 5th ed. Elsevier; 1973. p. 426–58.
 17. Colley EC, Stead RH. Optimized Immunohistochemistry Workflow Facilitated by New Dako Autostainer Link 48 Software Optimized Immunohistochemistry Workflow Facilitated by New Dako Autostainer Link 48 Software Abstract Introduction : In 2011. p. 1–8.
 18. Martinez-Ciarpaglini C, Fleitas-Kanonnikoff T, Gambardella V, Llorca M, Mongort C, Mengual R, et al. Assessing molecular subtypes of gastric cancer: Microsatellite unstable and Epstein-Barr virus subtypes. Methods for detection and clinical and pathological implications. *ESMO Open*. 2019;4(3):e000470.
 19. Lee HJ, Jang YJ, Lee EJ, Kim JH, Park SS, Park SH, et al. The significance of mismatch repair genes in gastric cancer. *J Cancer Res Ther* [Internet]. 2013 Jan 1;9(1):80–3. Available from: <https://www.cancerjournal.net/article.asp?issn=0973-1482>
 20. Hewitt LC, Inam IZ, Saito Y, Yoshikawa T, Quaas A, Hoelscher A, et al. Epstein-Barr virus and mismatch repair deficiency status differ between oesophageal and gastric

- cancer: A large multi-centre study. *Eur J Cancer*. 2018 May;94:104–14.
21. Haron NH, Hanif EAM, Manaf MRA, Yaakub JA, Harun R, Mohamed R, et al. Microsatellite instability and altered expressions of MLH1 and MSH2 in gastric cancer. *Asian Pacific J Cancer Prev*. 2019 Feb;20(2):509–17.
 22. Rawla P, Barsouk A. Epidemiology of gastric cancer: Global trends, risk factors and prevention. *Prz Gastroenterol*. 2019;14(1):26–38.
 23. Badary DM, Abdel-Wanis ME, Hafez MZ, Aboulhagag NA. Immunohistochemical analysis of PTEN, HER2/neu, and ki67 expression in patients with gastric cancer and their association with survival. *Pathophysiology*. 2017 Jun;24(2):99–106.
 24. Garattini SK, Basile D, Cattaneo M, Fanotto V, Ongaro E, Bonotto M, et al. Molecular classifications of gastric cancers: Novel insights and possible future applications. *World J Gastrointest Oncol*. 2017 May;9(5):194–208.
 25. Alessandrini L, Manchi M, De Re V, Dolcetti R, Canzonieri V. Proposed molecular and miRNA classification of gastric cancer. *Int J Mol Sci*. 2018 Jun;19(6).
 26. Seeneevassen L, Bessède E, Mégraud F, Lehours P, Dubus P, Varon C. Gastric cancer: Advances in carcinogenesis research and new therapeutic strategies. *Int J Mol Sci*. 2021 Mar;22(7).
 27. Birkman EM, Mansuri N, Kurki S, Ålgars A, Lintunen M, Ristamäki R, et al. Gastric cancer: immunohistochemical classification of molecular subtypes and their association with clinicopathological characteristics. *Virchows Arch*. 2018 Mar;472(3):369–82.
 28. Setia N, Agoston AT, Han HS, Mullen JT, Duda DG, Clark JW, et al. A protein and mRNA expression-based classification of gastric cancer. *Mod Pathol*. 2016 Jul;29(7):772–84.
 29. Richman S. Deficient mismatch repair: Read all about it (Review). *Int J Oncol*. 2015 Oct;47(4):1189–202.
 30. Doukas SG, Vageli DP, Nikolouzakis TK, Falzone L, Docea AO, Lazopoulos G, et al. Role of DNA mismatch repair genes in lung and head and neck cancer (Review). *World Acad Sci J [Internet]*. 2019;1(4):184–91. Available from: <https://doi.org/10.3892/wasj.2019.21>
 31. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. *J Clin Oncol*. 2019 Feb;37(4):286–95.
 32. Bae YS, Kim H, Noh SH, Kim H. Usefulness of immunohistochemistry for microsatellite instability screening in gastric cancer. *Gut Liver*. 2015 Sep;9(5):629–35.
 33. Kim KJ, Jung HY, Oh MH, Cho H, Lee JH, Lee HJ, et al. Loss of ARID1A Expression in Gastric Cancer: Correlation with Mismatch Repair Deficiency and Clinicopathologic Features. *J Gastric Cancer*. 2015 Sep;15(3):201–8.
 34. Chiaravalli AM, Furlan D, Facco C, Tibiletti MG, Dionigi A, Casati B, et al.

- Immunohistochemical pattern of hMSH2/hMLH1 in familial and sporadic colorectal, gastric, endometrial and ovarian carcinomas with instability in microsatellite sequences. *Virchows Arch* [Internet]. 2001;438(1):39–48. Available from: <https://doi.org/10.1007/s004280000325>
35. Hudler P. Genetic aspects of gastric cancer instability. Belkhiri A, editor. *Sci World J* [Internet]. 2012;2012:761909. Available from: <https://doi.org/10.1100/2012/761909>
 36. Koh J, Lee KW, Nam SK, Seo AN, Kim JW, Kim JW, et al. Development and Validation of an Easy-to-Implement, Practical Algorithm for the Identification of Molecular Subtypes of Gastric Cancer: Prognostic and Therapeutic Implications. *Oncologist*. 2019 Dec;24(12):e1321–30.
 37. Corti C, Sajjadi E, Fusco N. Determination of mismatch repair status in human cancer and its clinical significance: Does one size fit all? *Adv Anat Pathol*. 2019 Jul;26(4):270–9.
 38. Shia J. The diversity of tumours with microsatellite instability: molecular mechanisms and impact upon microsatellite instability testing and mismatch repair protein immunohistochemistry. *Histopathology*. 2021 Mar;78(4):485–97.
 39. Chen ML, Chen JY, Hu J, Chen Q, Yu LX, Liu BR, et al. Comparison of microsatellite status detection methods in colorectal carcinoma. *Int J Clin Exp Pathol* [Internet]. 2018;11(3):1431–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/31938240><http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6958115>
 40. Bass AJ, Thorsson V, Shmulevich I, Reynolds SM, Miller M, Bernard B, et al. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014 Sep;513(7517):202–9.
 41. Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med*. 2015 May;21(5):449–56.
 42. Díaz del Arco C, Estrada Muñoz L, Molina Roldán E, Cerón Nieto MÁ, Ortega Medina L, García Gómez de las Heras S, et al. Immunohistochemical classification of gastric cancer based on new molecular biomarkers: a potential predictor of survival. *Virchows Arch*. 2018 Dec;473(6):687–95.
 43. Di Pinto F, Armentano R, Arborea G, Schena N, Donghia R, Valentini AM. Are Immunohistochemical Markers Useful in Phenotypic Gastric Cancer Classification? *Oncol* [Internet]. 2020;98(8):566–74. Available from: <https://www.karger.com/DOI/10.1159/000506077>
 44. Pinto MP, Córdova-Delgado M, Retamal IN, Muñoz-Medel M, Bravo ML, Durán D, et al. A molecular stratification of Chilean gastric cancer patients with potential clinical applicability. *Cancers (Basel)*. 2020 Jul;12(7):1–14.
 45. Ito T, Suzuki O, Kamae N, Tamaru JI, Arai T, Yamaguchi T, et al. Comprehensive analysis of DNA mismatch repair-deficient gastric cancer in a Japanese hospital-based population. *Jpn J Clin Oncol*. 2021 May;51(6):886–94.

46. Sugimoto R, Endo M, Osakabe M, Toya Y, Yanagawa N, Matsumoto T, et al. Immunohistochemical Analysis of Mismatch Repair Gene Proteins in Early Gastric Cancer Based on Microsatellite Status. *Digestion*. 2021;102(5):691–700.
47. Verma R, Sakhuja P, Srivastava R, Sharma P. Implication of expression of MMR proteins and clinicopathological characteristics in gastric cancer. *Asia-Pacific J Oncol*. 2020 Dec 30;1–7.
48. Gonzalez RS, Messing S, Tu X, McMahon LA, Whitney-Miller CL. Immunohistochemistry as a surrogate for molecular subtyping of gastric adenocarcinoma. *Hum Pathol*. 2016 Oct;56:16–21.
49. Ahn S, Lee SJ, Kim Y, Kim A, Shin N, Choi KU, et al. High-throughput protein and mRNA expression-based classification of gastric cancers can identify clinically distinct subtypes, concordant with recent molecular classifications. *Am J Surg Pathol*. 2017 Jan;41(1):106–15.
50. Tsai CY, Lin TA, Huang SC, Hsu JT, Yeh CN, Chen TC, et al. Is Adjuvant Chemotherapy Necessary for Patients with Deficient Mismatch Repair Gastric Cancer?-Autophagy Inhibition Matches the Mismatched. *Oncologist*. 2020 Jul;25(7):e1021–30.
51. Arai T, Sakurai U, Sawabe M, Honma N, Aida J, Ushio Y, et al. Frequent microsatellite instability in papillary and solid-type, poorly differentiated adenocarcinomas of the stomach. *Gastric Cancer*. 2013 Oct;16(4):505–12.
52. Sun Y, Yu W, Guan W, Cai L, Qiao M, Zheng L, et al. Integrated assessment of PD-L1 expression and molecular classification facilitates therapy selection and prognosis prediction in gastric cancer. *Cancer Manag Res*. 2019;11:6397–410.
53. Setia N, Ahn S, Han HS, Park DY, Lauwers GY. Predictive value of WHO classification for PD-L1 and Her2/Neu expression and distinct associations with protein expression based classification in gastric carcinoma. *Hum Pathol*. 2019 Dec;94:64–70.
54. GamzeErkılınç ŞirinBaşpınar, Zümürüt ArdaKaymak, ŞehnazEvrimler N. HATALI EŞLEŞME GENLERİNDEN MLH1, PMS2, MSH6, MSH2'İN MİDE KANSERLERİNDE İMMÜNHİSTOKİMYASAL EKSPRESYONU; BİR DOKU MİKROARRAY ÇALIŞMASI. *Journal*. 2021;28(3):487–97.
55. Shin SJ, Kim SY, Choi YY, Son T, Cheong JH, Hyung WJ, et al. Mismatch Repair Status of Gastric Cancer and Its Association with the Local and Systemic Immune Response. *Oncologist*. 2019 Sep;24(9):e835–44.
56. Ramos MFKP, Pereira MA, de Mello ES, Cirqueira CDS, Zilberstein B, Alves VAF, et al. Gastric cancer molecular classification based on immunohistochemistry and in situ hybridization: Analysis in western patients after curative-intent surgery. *World J Clin Oncol*. 2021 Aug;12(8):688–701.
57. Pereira MA, Ramos MFKP, Faraj SF, Dias AR, Yagi OK, Zilberstein B, et al. Clinicopathological and prognostic features of Epstein-Barr virus infection, microsatellite instability, and PD-L1 expression in gastric cancer. *J Surg Oncol*. 2018 Apr;117(5):829–39.

58. Yoon JY, Sy K, Brezden-Masley C, Streutker CJ. Histo- and immunohistochemistry-based estimation of the TCGA and ACRG molecular subtypes for gastric carcinoma and their prognostic significance: A single-institution study. *PLoS One*. 2019;14(12):e0224812.
59. Nshizirungu JP, Bennis S, Mellouki I, Sekal M, Benajah DA, Lahmidani N, et al. Reproduction of the Cancer Genome Atlas (TCGA) and Asian Cancer Research Group (ACRG) Gastric Cancer Molecular Classifications and Their Association with Clinicopathological Characteristics and Overall Survival in Moroccan Patients. *Dis Markers*. 2021;2021:9980410.
60. Zhang Q, Wang L, Ni S, Tan C, Cai X, Huang D, et al. Clinicopathological features and prognostic value of mismatch repair protein deficiency in gastric cancer. *Int J Clin Exp Pathol*. 2018;11(5):2579–87.
61. Yamamoto H, Imai K. Microsatellite instability: an update. *Arch Toxicol*. 2015 Jun;89(6):899–921.
62. Deschoolmeester V, Baay M, Van Marck E, Weyler J, Vermeulen P, Lardon F, et al. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. *BMC Immunol*. 2010 Apr;11:19.
63. Rohilla KJ, Gagnon KT. RNA biology of disease-associated microsatellite repeat expansions. *Acta Neuropathol Commun* [Internet]. 2017;5(1):63. Available from: <https://doi.org/10.1186/s40478-017-0468-y>
64. Kim JY, Kim WG, Kwon CH, Park DY. Differences in immune contexts among different molecular subtypes of gastric cancer and their prognostic impact. *Gastric cancer Off J Int Gastric Cancer Assoc Japanese Gastric Cancer Assoc*. 2019 Nov;22(6):1164–75.
65. Bykov VJN, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. *Nat Rev Cancer*. 2018 Feb;18(2):89–102.
66. Demir Ö, Barros EP, Offutt TL, Rosenfeld M, Amaro RE. An integrated view of p53 dynamics, function, and reactivation. *Curr Opin Struct Biol* [Internet]. 2021;67:187–94. Available from: <https://www.sciencedirect.com/science/article/pii/S0959440X20302013>
67. Ando K, Oki E, Saeki H, Yan Z, Tsuda Y, Hidaka G, et al. Discrimination of p53 immunohistochemistry-positive tumors by its staining pattern in gastric cancer. *Cancer Med*. 2015 Jan;4(1):75–83.
68. Fenoglio-Preiser CM, Wang J, Stemmermann GN, Noffsinger A. TP53 and gastric carcinoma: a review. *Hum Mutat*. 2003 Mar;21(3):258–70.
69. Grosser B, Kohlruss M, Slotta-Huspenina J, Jesinghaus M, Pfarr N, Steiger K, et al. Impact of Tumor Localization and Molecular Subtypes on the Prognostic and Predictive Significance of p53 Expression in Gastric Cancer. *Cancers (Basel)*. 2020 Jun;12(6).
70. Hwang HJ, Nam SK, Park H, Park Y, Koh J, Na HY, et al. Prediction of TP53 mutations by p53 immunohistochemistry and their prognostic significance in gastric cancer. *J Pathol Transl Med*. 2020 Sep;54(5):378–86.

71. Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih IM, et al. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: An immunohistochemical and nucleotide sequencing analysis. *Mod Pathol*. 2011;24(9):1248–53.
72. Köbel M, Rahimi K, Rambau PF, Naugler C, Le Page C, Meunier L, et al. An Immunohistochemical Algorithm for Ovarian Carcinoma Typing. *Int J Gynecol Pathol Off J Int Soc Gynecol Pathol*. 2016 Sep;35(5):430–41.
73. Daun T, Nienhold R, Paasinen-Sohns A, Frank A, Sachs M, Zlobec I, et al. Combined Simplified Molecular Classification of Gastric Adenocarcinoma, Enhanced by Lymph Node Status: An Integrative Approach. *Cancers (Basel)*. 2021 Jul;13(15).
74. Kim BH, Kóvári B, Kim H, Boulware DC, Pimiento J, Lauwers GY. Comparative molecular subtypes of index and metachronous gastric adenocarcinomas: a study of 42 Korean patients. *Mod Pathol an Off J United States Can Acad Pathol Inc*. 2021 Sep;34(9):1728–37.
75. Kim HS, Shin SJ, Beom SH, Jung M, Choi YY, Son T, et al. Comprehensive expression profiles of gastric cancer molecular subtypes by immunohistochemistry: implications for individualized therapy. *Oncotarget*. 2016 Jul;7(28):44608–20.
76. Gurzu S, Jung I, Sugimura H, Stefan-van Staden RI, Yamada H, Natsume H, et al. Maspin subcellular expression in wild-type and mutant TP53 gastric cancers. *World J Gastrointest Oncol*. 2020 Jul;12(7):741–55.