

The Correlation of P53 and MSI Immune Markers in Gastric Adenocarcinoma

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Abstract:

Background: The known risk factors for gastric adenocarcinoma are chromosomal instability, TP53 mutations, aneuploidy, translocations, proto-oncogenes, and tumor suppressor gene changes. Microsatellite instability (MSI) affects DNA replication accuracy and is detected by the heterodimeric protein complex hMSH2/hMSH6, which recruits hMLH1 and hPMS2 for re-synthesis. MSI can cause sporadic gastric cancer and Lynch syndrome.

Objectives: To examine the relationship between P53 and MSI immune markers expression with the clinicopathological parameters of gastric adenocarcinoma by using immunohistochemistry.

Methods: The study examined 40 formalin-fixed, paraffin-embedded gastric adenocarcinoma tissue blocks. The samples were retrieved from archived materials in the histopathology department of the Gastroenterology and Hepatology Teaching Hospital, Teaching Laboratory Institute, and some private laboratories in Baghdad, Iraq. The samples were taken from patients between 2020 and 2023, while their retrieval spanned from October 2022 to October 2023 for the sake of examining primary cases, surgical tissues, and available clinicopathological data. The immunohistochemical (IHC) expression was assessed using a scoring system. Data were analyzed using SPSS, Chi-square, and Fisher's exact tests, with a 95% confidence level and a 0.05 P-value or less considered significant.

Results: IHC staining for P53 was positive in 65% of the samples, while MSI findings were positive in 97.5% of the samples. The MLH1/PMS2 heterodimeric couple showed 32.5% positive results, while the MSH2/MSH6 heterodimeric couple showed 87.5% positive results. P53 stain was significantly correlated with lymph node involvement and grade, but not with the other parameters. No significant association was found between MSI markers and the studied parameters. There was no significant association between MSI heterodimeric couple (MLH1/PMS2) and the clinicopathological parameters, but there was a significant association between MSI heterodimeric couple (MSH2/MSH6) markers and metastasis only.

Conclusion: P53 is a key biomarker for evaluating lymph node involvement and aggressiveness in grading, indicating prognosis, and identifying high-risk cancer patients for metastasis.

Keywords: Gastric adenocarcinoma; molecular classification; immunohistochemistry; P53; microsatellite instability.

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Introduction:

The Global Cancer Observatory (GLOBOCAN) in 2020 indicated that stomach cancer was the fifth largest cause of cancer deaths, with 1.1 million cases of which 75% were in Asia. Five-year survival is 20%, with Eastern Asians having the greatest incidence (22.4 per 100,000) (1,2). Stomach cancer, which is Iraq's second-leading cause of cancer mortality, killed 783,000 people worldwide in 2018 and caused over 1,000,000 new cases with clinical, genetic, morphological, epidemiological, and developmental abnormalities (3). It is the fifth most prevalent cancer and the third leading cause of cancer death worldwide, caused by environmental and genetic factors, including *Helicobacter pylori*. [4]. GLOBOCAN 2021 reported Iraqi stomach cancer incidence, death, and prevalence as follows: New cases (all ages) 1149 (3.4%) with a rank of 9

and a cumulative risk of 0.56. There were 966 deaths (4.9%) with a rank of 6 and a cumulative risk of 0.48. Presence at 5 years was 1579, or 3.39 per 100,000 (5). Most non-cardia stomach cancers are caused by *H. pylori*, the first bacterial carcinogen. Diffuse gastric cancer, non-cardia intestinal gastric adenocarcinoma, and gastric B-cell lymphocyte mucosa-associated lymphoid tissue lymphoma can result from childhood acquisition (6). As *H. pylori* eradication may restore atrophic gastritis but not intestinal metaplasia, targeted intervention before stomach precancerous changes may help high-risk individuals prevent gastric cancer (6). Among the factors that increase the risk of gastric cancer are dietary nitrite-secondary amines, high-temperature cooking of protein-rich foods, high salt intake, smoking, and excessive alcohol consumption. On the other hand, Vitamin C, onions, garlic, and shallots reduce stomach carcinogenesis (6).

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Hereditary diffuse gastric cancer, BRCA2, HNPCC, Lynch II, Li Fraumeni syndrome, and FAP syndrome increase stomach cancer risk (6). The 2019 WHO categorization of malignant epithelial tumors includes tubular, papillary, poorly cohesive signet ring phenotype, poorly cohesive other cell type, mucinous, and mixed histologic types (7). Intestinal and diffuse gastric cancer subtypes have diverse shapes, epidemiology, pathogenic mechanisms, and genetic profiles. Intestinal tumors are tubular or glandular, with better prognosis in males and older individuals. Poorly cohesive carcinomas invade glandular structures (8). The Cancer Genome Atlas (TCGA) reveals that 20% of stomach cancers are genetically stable (GS), aneuploid, and early-identified, with 73% diffuse subtype enrichment, cadherin-1(CDH1) somatic mutations (8-10). Fifty percent of stomach cancers are chromosomally unstable (CIN), more common in esophageal gastric junction tumors. P53, a cell cycle regulator, prevents DNA replication errors during synthesis, minimizing cancer progression. Mutation or heterozygosity loss usually inactivates it on 17p. (11). TP53 mutations and histological P53 overexpression are important molecular factors in understanding stomach cancers, as trastuzumab can treat 10–20% of gastric adenocarcinomas if HER2 is overexpressed [8]. MSI results from DNA mismatch repair deficiencies. The Mismatch Repair (MMR) system—hMLH1, hMSH2, hMSH6, and hPMS2 proteins—corrects base mismatches, insertions, and deletions for DNA replication accuracy (8). Lynch syndrome, caused by autosomal dominant MMR gene defects, increases cancer risk at younger ages, particularly in colorectal, endometrial, ovarian, and gastric cancers. Microsatellite-unstable gastric tumors, accounting for 22% of cases, have a high mutation rate (8). Epstein-Barr, a herpes virus, infects B-cells in the oropharyngeal epithelium, leading to various cancers like breast, lung, stomach, colon, and lung, due to complex interactions between cell environment and viral gene expression. (12). EBV gene expression and host genome control impact oncogenesis, affecting stomach cancer's host gene expression and cell cycle pathways. Recent research links viral latent profiles to latency I or II. EBV-positive gastric tumors, primarily affecting men, have the best prognosis, with genetically stable subtypes having the worst prognosis (8,13). The Asian Cancer Research Group (ACRG) found four molecular categories for gastric cancer. The second classification algorithm firstly includes the mesenchymal group with microsatellite stability (MSS) and epithelial-mesenchymal transition (EMT), which accounts for 15.3% of cases and is usually found in advanced stages. In 80% of cases, signet ring cell carcinomas occur. The second MSS/TP53-negative subtype accounts for 35.7% of cases, while the third subtype MSS/TP53+ positive which has more EBV infections. The fourth subtype, microsatellite unstable, starts in the distal stomach and has the best prognosis (8).

Patients, Materials and Methods:

Our study was conducted on 40 stomach cancer patients using formalin-fixed paraffin-embedded tissue blocks. The samples were collected from the Gastroenterology and Hepatology Teaching Hospital, Teaching Laboratory Institute, and private laboratories between 2020 and 2023. After possibly curative gastrectomy and lymphadenectomy, the patients were histologically divided into 19 intestinal, 14 diffuse, and 7 mixed adenocarcinoma groups. The study focused on cases with primary gastric adenocarcinoma, available clinicopathological data, and surgical specimens with available tissue for paraffin blocks. Immunological markers were investigated, including P53 and MSI (MLH1, MSH2, MSH6, and PMS2). The study was performed in a private laboratory and excluded other gastric tumors, secondary gastric adenocarcinoma, endoscopic biopsies, and gastric cancers with pre-operative neoadjuvant therapy.

Table 1: Materials

Material	Type
Xylene	Analar (England)
Ethanol (absolute)	Merck (Germany)
Distilled water	
Rinse buffer	TBS (DakoCytomation)
Target retrieval solution (heat-induced epitope retrieval (HIER) DAKO PT LINK (code PT100/PT101)	Tris EDTA pH 9.0 (Dakocytomation) EnVision FLEX Target retrieval solution HIGH pH 50x code (K8000 /K8004)
Primary antibody	DAKO FLEX monoclonal mouse anti-human p53 protein (clone DO-7). Isotype: IgG2b, kappa. Ready-to-use (Link) Code IR616 DAKO FLEX monoclonal mouse Anti-Epstein-Barr Virus, LMP, (Clone CS.1-4). Isotype: IgG1, kappa. Ready-to-use (Link) Code IR753 DAKO FLEX monoclonal mouse Anti-Human E-Cadherin, (clone NCH-38) Isotype: IgG1, kappa. Ready-to-use (Link), Code IR059 DAKO MLH1 Clone ES05 Ready-to-use (Prediluted) Product no/lot no.: IR079/IS079/ 11450820 DAKO MSH2 Clone FE11 Ready-to-use (Prediluted) Product no/lot no.: IR085 / 10148024 DAKO MSH6 Clone EP49 Ready-to-use (Prediluted) Product no/lot no.: IR086 / 11166400 DAKO PMS2 Clone EP51 Ready-to-use (Prediluted) Product no/lot no.: IR087 / 11170264
Hematoxylin	Counter stain EnVision FLEX Hematoxylin (link) (code K80008)
Mounting media	Dakocytomation
Secondary detection system	HRP/DAB detection (Dakocytomation)
Visualization system	EnVision FLEX High pH (Link) (code K8000) for p53, EBV. EnVision FLEX+ mouse High pH (Link) (code K8002) for E-CADHERIN, MLH1, MSH2, MSH6 and PMS2

Methods: The process involved deparaffinizing blocks in an oven at 60°C for 1 hour, followed by xylene swaps. The tissue was rehydrated with ethanol, and a hematoxylin nuclear stain was applied. Differentiation was done in a 1% acid-alcohol solution, and eosin counterstain was used. Mounting was done using DPX, and H&E slides were examined to choose the best sections for IHC. The immunohistochemistry process involved sectioning tissue blocks, incubating them in a water bath, deparaffinization, applying Xylene, rehydration in alcohol solutions, and rinsing with tap water. The antigen retrieval phase involved using a tris ethylenediamine tetra-acetic acid (TRIS EDTA) solution heated to 80°C and maintained for 20 minutes before being lowered back to 65°C for each cycle. A PAP pen was used as a reagent blocker, and a wash buffer solution was used. Two drops of peroxidase blocker were applied to stop the endogenous antigen activity. Primary antibodies were applied to the samples, targeting five markers: P53, MLH1, MSH2, MSH6, and PMS2, and each was incubated for 30 minutes. Anti-mouse and anti-rabbit antibodies labeled with horseradish peroxidase were applied and washed. (3,3-diaminobenzidine) DAB was prepared by adding poly detector DAB chromogen per milliliter of poly detector DAB buffer. The samples were washed with wash buffer, and hematoxylin counterstain was applied to the background for one minute. For the MSI markers, Gastric cancer is considered negative if no tumor cell staining is present for all the markers, and tumors with all markers' expression are considered microsatellite stable (14), while other articles suggested that the loss of expression of a single protein or a heterodimeric couple supports MMRD, which is indirect evidence of MSI. Proteins hMLH1 and hMSH2 are stable without their dimeric partners, hPMS2 and hMSH6, but these components are rarely stable (15).

For the quality control, basal epithelial cells in the colon and appendix show a moderate to strong staining reaction, while germinal centers in cells of the tonsil show a moderate to strong staining reaction; both are considered positive controls for MLH1, MSH2, MSH6, and PMS2. Colonic adenocarcinoma with loss of MLH1, MSH2, MSH6, and PMS2 expression can serve as a negative control, and stromal cells show a distinct nuclear staining reaction serving as an internal positive tissue control, as mentioned in the antibody leaflet. The expression of P53 cells is typically detected through nuclear staining. Two patterns are considered abnormal: Strong nuclear staining in at least 70% of tumor cells and complete loss of p53 expression, or less than 5%. Stromal cells and benign epithelium served as controls for normal and reactive mesothelium, with mesotheliomas showing negative cells (14). Neoplastic cells of colonic adenocarcinoma with a moderate to strong staining reaction were considered a positive control, and normal colonic mucosa was considered a negative control, as mentioned in the

antibody leaflet. The interpretation of the slides and the correlation of the immune markers' expression and the clinicopathological parameters: Age, sex, location of the tumor, type of surgery, morphological tumor pattern, TNM staging, tumor grade, lympho-vascular invasion, and perineural invasion were done by the authors.

Statistical analysis: The study used Statistical Package for Social Sciences (SPSS) version 26 to describe variables, with serial numbers being the only reference for participant details. Data were managed daily and expressed using mean, standard deviation, and frequency/ percentage. The Chi-square and Fisher's exact tests were used to assess the association between categorical variables, with a 95% confidence level and a P-value of 0.05 or less being considered significant.

Results

The study examined 40 cases of gastric adenocarcinoma, with 57.5% being males and 42.5% being females. The age distribution of the patients showed that one case (2.5%) was between 20-29 years of age, 6 cases (15%) were between 30-39 years, 10 cases (25%) were between 40-49 years, 10 cases (25%) were between 50-59 years, and 13 cases (32.5%) were 60 years or over.

The samples were from the proximal stomach gastroesophageal junction and cardia (10%), (2.5%) in the fundus, (50%) in the body and antrum, and (37.5%) in the distal stomach. The cases were treated with total gastrectomy (62.5%), proximal gastrectomy (5%), and distal gastrectomy (32.5%). There were 19 cases (47.5%) of intestinal type adenocarcinoma, 14 cases (35%) of diffuse type adenocarcinoma, and 7 cases (17.5%) of mixed type adenocarcinoma, (table 2). Four stages were identified: 1A and 1B, 2A and 2B, 3A and 3B, and 4, (table 4). The cases were graded into G1 well-differentiated adenocarcinoma, G2 moderately differentiated adenocarcinoma, G2/G3 moderately to poorly differentiated adenocarcinoma, and G3 poorly differentiated adenocarcinoma. Only 17). 42.5% of the cases showed lympho-vascular invasion, and 18. While 45% showed perineural invasion.

Table 2: Distribution of the samples by site, specimen and diagnosis

Variable	Category	NO.	%
Site	Proximal stomach gastroesophageal junction and cardia	4	10.0
	Fundus	1	2.5
	Body and antrum	20	50.0
	Distal stomach	15	37.5
Specimen	Total gastrectomy	25	62.5
	Proximal gastrectomy	2	5.0
	Distal gastrectomy	13	32.5
Diagnosis	Intestinal type adenocarcinoma	19	47.5
	Diffuse type adenocarcinoma	14	35.0
	Mixed type adenocarcinoma	7	17.5

Table 4: Distribution of the samples by stage and grade

Variable	Category	NO.	%
Stage	1A	1	2.5
	1B	5	12.5
	2A	5	12.5
	2B	11	27.5
	3A	6	15.0
	3B	10	25.0
	4	2	5.0
T	1	2	5.0
	2	7	17.5
	3	26	65.0
	4A	4	10.0
	4B	1	2.5
N	0	11	27.5
	1	9	22.5
	2	8	20.0
	3	4	10.0
	3A	4	10.0
	3B	2	5.0
	X	2	5.0
	0	1	2.5
M	1	2	5.0
	X	37	92.5
	0	1	2.5
Grade	G1 well differentiated	1	2.5
	G2 moderately differentiated	24	60.0
	G2/G3 moderately to poorly differentiated	2	5.0
	G3 poorly differentiated	13	32.5

The study found that P53 was positive in 26 cases (65%) and MSI was positive in 39 samples (97.5%). Immune markers as heterodimeric couples were found to be positive as follows: MHL1/PMS2 heterodimeric couple (32.5%) of the samples, MSH2/MSH6 heterodimeric couple (87.5%) of the samples, and 35% of the samples were found to be positive for P53 and negative for MSI expression, tables 4 and 5

Table 4: Distribution of the samples by the main stains

Variable	Category	Number	%
P53	Positive	26	65.0
	Negative	14	35.0
MSI	Positive	39	97.5
	Negative	1	2.5

Table 5: Distribution of the samples by the stain couples

Variable	Category	Number	%
MLH1/PMS2	Positive	13	32.5
	Negative	27	67.5
MSH2/MSH6	Positive	35	87.5
	Negative	5	12.5

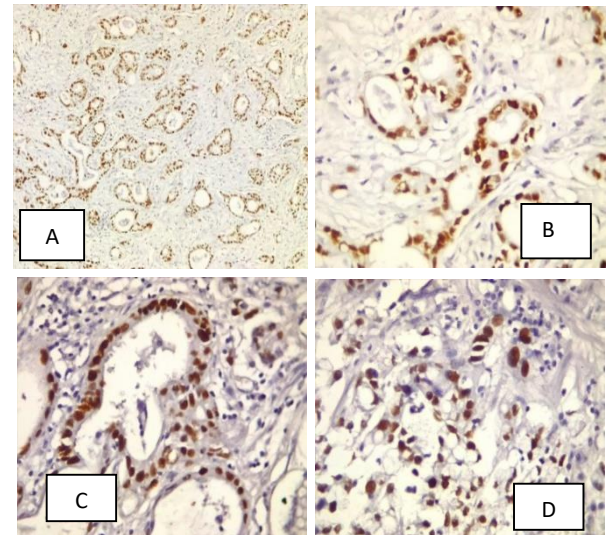


Figure (6): P53 positive immune markers overexpression

- A: P53 positive nuclear staining >70 % overexpression (40x)
- B, C: P53 positive nuclear staining >70 % overexpression (100x)
- D: P53 positive nuclear staining >70 % overexpression (400x)

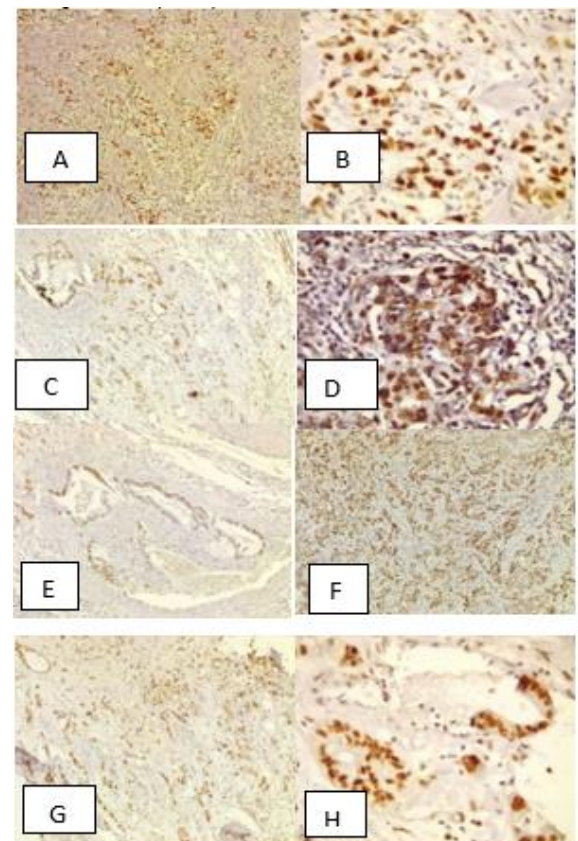


Figure (7): MSI immune markers positive expression

- A: MLH1 positive 100x, B: MLH1 positive 400x
- C: MSH2 positive 100x, D: MSH2 positive 400x
- E: MSH6 positive 100x, F: MSH6 positive 400x
- G: PMS2 positive 100x, H: PMS2 positive 400x

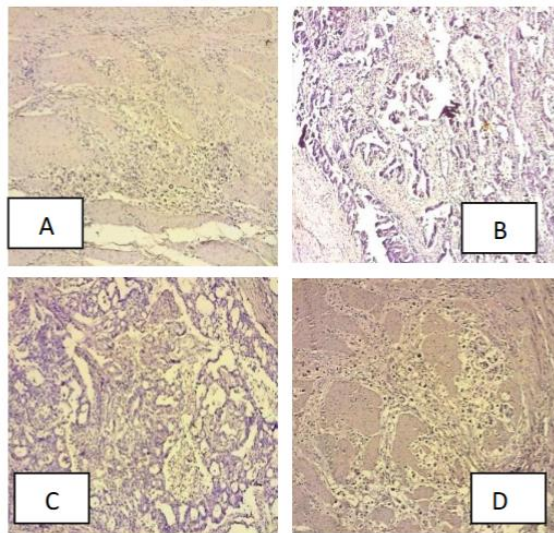


Figure (8): MSI immune markers negative expression

A: MLH1 negative 100x
B: MSH2 negative 100x
C: MSH6 negative 100x
D: PMS2 negative 100x.

A significant association was found between the P53 score and lymph node involvement ($P = 0.047$) and grade ($P = 0.012$), table (5). There was no significant association between the P53 score and age group ($P = 0.135$), sex ($P = 0.191$), site of the tumor ($P = 0.245$), specimen ($P = 0.754$), diagnosis ($P = 0.677$), stage ($P = 0.124$), tumor size ($P = 0.371$), metastasis ($P = 0.693$), lymphovascular ($P = 0.973$), or perineural invasion ($P = 0.257$).

Table 5: Distribution of P53 stain by the sample characteristics

Variable	Category	P53				P value
		Positive	Negative	No. = 26	No. = 14	
N	0	8	3	30.8	21.4	0.047* ¹
	1	3	6	11.5	42.9	
	2	7	1	26.9	7.1	
	3	4	0	15.4	0.0	
	3A	3	1	11.5	7.1	
	3B	1	1	3.8	7.1	
	X	0	2	0.0	14.3	
Grade	G1 well differentiated	0	1	0.0	7.1	0.012* ¹
	G2 moderately differentiated	12	12	46.2	85.7	
	G2/G3 moderately to poorly differentiated	2	0	7.7	0.0	
	G3 poorly differentiated	12	1	46.2	7.1	

*Significant result

¹Fisher's exact test

There was no significant association between MSI markers when they were considered as one marker and age group, sex, site of the tumor, specimen, diagnosis, stage, tumor size, metastasis, lymph nodal involvement, tumor grade, lympho-vascular, and perineural invasion ($P > 0.05$). As for the heterodimeric MSI couple (MLH1/PMS2) with the clinicopathological parameters, there was no (MLH1/PMS2) and age group, sex, site of the tumor, specimen, diagnosis, stage, tumor size, metastasis, lymph node involvement, tumor grade, lympho-vascular, and perineural invasion ($P > 0.05$). For the significant association between MSI markers when they were considered as a heterodimeric couple heterodimeric MSI couple (MSH2/MSH6) relationship with the clinicopathological parameters, there was a significant association between MSI markers when they were considered as a heterodimeric couple (MSH2/MSH6), and metastasis

($P = 0.036$), table (6). No significant association was found between MSI markers when they were considered as a heterodimeric couple (MSH2/MSH6), and age group, sex, site of the tumor, specimen, diagnosis, stage, tumor size, lymph node involvement, tumor grade, lymphovascular, and perineural invasion ($P > 0.05$).

Table 6: Distribution of MSH2/MSH6 stain by sample characteristics

Variable (M)	MSH2/MSH6				P value
	Positive		Negative		
	N=35	%	N=5	%	
0	0	0.0	1	20.0	0.036*
1	1	2.9	1	20.0	
X	34	97.1	3	60.0	

*Significant result Fisher's exact test used

Discussion

The results of the current study agree with the study of Al-Badri et al, who found a significant association between p53 expression and tumor grade and lymph node involvement in gastric carcinoma and gastric dysplasia. However, no significant association was found between p53 protein expression and tumor depth or histological type. [11]. Grosser et al found that abnormal p53 expression negatively impacts patients' prognosis in resection specimens. The study found that P53 did not predict response or survival in the biopsy cohort before CTx. The expression of P53 varied across molecular subtypes in surgical resection and biopsied specimens, with a clear correlation between P53 and MSI-L. Individuals with MSI-H and abnormal P53 had the worst survival outcomes in biopsy patients. Our results are in disagreement with these results as they found a relationship between p53 and MSI expression that had the worst survival outcome which we didn't investigate. [16]. Hwang et al conducted a study using deep-targeted sequencing on surgical or biopsy materials from 120 individuals with gastric cancer. They found that high P53 expression was linked to TP53 missense mutations, negative expression was related to other mutations, and weak expression was seen in cases with wild-type TP53.

The preliminary diagnostic TNM staging showed a strong association with both TP53 mutation type and P53 expression status. A survival study on 109 stage II and III gastric cancer cases revealed that patients with TP53 missense mutations had significantly worse overall survival compared to wild-type and other mutation groups. A higher level of P53 expression was associated with a worse overall survival rate. For the comparison with our study, we partially agree because we used IHC to examine the protein product and found a significant relation to lymph nodal involvement and grading which gave a prognostic insight, while in the comparative study, they used gene sequencing and found a relation of p53 expression to TP53 missense mutation which was an important poor prognostic factor and worse overall survival rate. [17]. A study by Kim et al found that among 3608 gastric cancer patients, 37% had P53 overexpression. In intestinal-type gastric cancer, overexpression was associated with less invasion depth and early-stage disease. In diffuse-type gastric cancer, overexpression was linked to advanced TNM stage and advanced disease. Patients with P53 overexpression had reduced overall survival and gastric cancer-specific survival, with the significance being more prominent in diffuse-type gastric cancer [18]. Zhang et al in a study on gastric

cancer found a significant positive correlation between Her-2 and P53 expression. The study found that Her-2 expression intensity varied significantly in patients with varying degrees of gastric cancer cell differentiation, with signet-ring cell carcinoma being strongly associated with Her-2 expression. The proportion of positive P53 expression was correlated with tumor differentiation grade and positive Ki67 expression, suggesting that HER-2 and P53 collaborate in gastric cancer. The study revealed a significant correlation between positive P53 expression, age, tumor differentiation grade, and Ki67 expression, with significant differences observed across groups with higher differentiation degrees, and a positive correlation between high P53 expression and poor differentiation [19]. Regarding the MSI immune stain reaction. The study of Karpínska-Kaczmarczyk et al on 107 patients with gastric cancer found an MSI deficit, with 5.6% of the patients showing MMR proteins. The loss of MMR protein expression was linked to intestinal gastric cancer in the Lauren classification and tubular and papillary architecture in the WHO classification. Negative MMR expression was not associated with age, sex, tumor site, depth of invasion, lymph node status, ulceration, or lymphocytic infiltration. Our results are not in agreement with these results as the mismatch repair protein expression does not show any correlation with the histological types [20]. Hanon et al in a study in Baghdad, Iraq, focusing on the prevalence of MSI in colorectal carcinoma (CRC) found that MSI prevalence was higher in women (38.1%) and older individuals (34.6%). Morphological features of CRC specimens showed a higher percentage (47.1%) in poorly differentiated cases. Mucinous CRC had 100% MSI compared to 27.7% for non-mucinous cases. MSI was more common at the right site (52.9%) than in MSI L and MSS, Hanon et al used PCR to study the MSI profile in colorectal carcinoma, while we used IHC for MMR proteins to assess gastric adenocarcinoma. [21]. Hiroki in a study on Japanese patients with early gastric malignancies found 54 adenocarcinomas, including high-grade dysplasia, treated with endoscopic resection over five years. The WHO characteristics re-evaluation revealed that EBV-positive carcinomas were poorly differentiated (83.8%), while MSI-H tumors were common in well-to moderately differentiated adenocarcinomas (85.7%). This highlights the importance of understanding the WHO criteria in subdividing Japanese early gastric malignancies, which may help compare precursor lesions and early carcinoma.

Significant differences in macroscopic characteristics and histological subtypes were observed between these groups, which was not relevant in our study. [22]. Reitsam et al found that 1.4% and 5.1% of patients with dMMR had loss of MLH1, PMS2, and MSH6 immuno-expression. The study examined MLH1 promotor hypermethylation and BRAF exon 15 status and sequenced DNA repair genes using next-generation sequencing. Pathogenic germline variants and sporadic mutations were identified in the MMR and HRR genes, affecting ATM, BARD1, BRCA1, CDK12, CHEK1, CHEK2, FANCA, MLH1, MSH6, PALB2, and TP53. This study considers the biological function of MMR proteins and next-generation sequencing as potential drug targets and the low frequency of most of these mutations in the digestive system which was not in the capability of our scope. [23]. Evaristo et al found that 12.3% of gastroesophageal junction tumors had the MMR-deficient (dMMR) immunophenotype, with most cases lacking the BRAF V600E mutation. The dMMR phenotype was not significantly associated with tumor grade but was associated with lower pathologic staging than the pMMR. Patients with pMMR tumors had a higher median number of positive lymph nodes than those with DMMR tumors, leading to higher pathologic lymph node staging groups which was irrelevant in our investigation regarding lymph nodal staging [24]. Zhang et al found that mismatch repair-deficient gastric cancer patients have higher programmed death ligand-1 expression and a higher incidence of MSI. They found 126 (6%) MLH1/PMS2-negative individuals and 14 (0.9%) MSH2/MSH6-negative ones. The study found a high association between d-MMR status and intestinal group, but not with the tumor differentiation which was irrelevant in our investigation regarding histological subtyping and differentiation (25). Elrefaey et al in a study in Egypt, examined the IHC expression of MLH1, MSH2, and P53 proteins to correlate them with tumor differentiation, lymph node status, and TNM staging in 70 gastric adenocarcinomas. They found a significant correlation between the MSI status and tumor differentiation, invasion depth, lymph node status, and TNM staging, while in our investigation, we only had a significant association between MSH2/MSH6 expression and metastasis [26]. A quick reference to the studies that tested other markers related to gastric adenocarcinoma, Ashour et al found a significant correlation between MUC5AC expression and lymph node involvement in gastric cancer patients, with a decrease in expression compared to the control group. However, there was no significant correlation between MUC5AC expression and age, sex, histopathological subtypes, grade, and stage of gastric cancer. The results suggest that MUC5AC can be used as an ancillary marker for diagnosing lymph node involvement and malignant transformation of gastric cancer, but not for predicting grade and stage outcomes [4]. Mwafaq et al found that there were significant differences in PARP1 expression levels

between patients and control groups, with significant correlations between histopathological subtype, grade, invasion depth, lymph node involvement, and stages in patients. However, no significant associations were found with age or sex [3]. These two later studies throw a light on the other IHC markers expression that was significantly correlated with some of the clinicopathological parameters so as P53 and mismatch repair proteins that we were interested in our research. The disagreement in all the mentioned studies above was regarded due to the small sample size, different methodology, different antibody clones, and subjective interpretation of the immune markers' expression.

Conclusions

P53 is a key biomarker for evaluating lymph node involvement and aggressiveness in grading, indicating prognosis, and identifying high-risk cancer patients for metastasis.

Authors' declaration:

There were no conflicts of interest. All of the tables in the text are original works by the authors. Authors signed off based on their acceptance of ethical considerations. The research ethical committee in the pathology and forensic medicine department (college of medicine/university of Baghdad) gave their stamp of approval to the study, according to the code No.145 (4THOCT-2022).

Author Contributions:

Study conception & design: (Ali M.J Al-Shakarchi & Sazan A.W. Mirza). Literature search: (Ali M.J Al-Shakarchi). Data acquisition: (Ali M.J Al-Shakarchi). Data analysis & interpretation: (Ali M.J Al-Shakarchi & Sazan A.W. Mirza). Manuscript preparation: (Ali M.J Al-Shakarchi). Manuscript editing & review: (Ali M.J Al-Shakarchi & Sazan A.W. Mirza).

References

1. Ilic M, Ilic I. Epidemiology of stomach cancer. *World journal of gastroenterology*. 2022;28(12):1187. <https://doi.org/10.3748/wjg.v28.i12.1187>
2. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. *International journal of cancer*. 2021;149(4):778-89. <https://doi.org/10.1002/ijc.33588>
3. Mwafaq RK, Abbas AK, Abdullah LAH. Evaluation of PARP-1 by imm unohistochemistry in a sample of Iraqi patients with gastric cancer. *Iraqi Journal of Science*. 2022;467-79. <https://doi.org/10.24996/ijc.2022.63.2.5>
4. Ashour HJ, Al-Bakri NA, Abdul Ghafour KH. The Immunohistochemical Assessment of Muc5ac in Patients with Gastric Carcinoma (Gc) in Iraq. *IJS*. 2019;460-8. <https://doi.org/0.24996/ijc.2019.60.3.6>

5. Observatory gc. global cancer observatory 2023. <https://gco.iarc.fr/today>
6. Toh JW, Wilson RB. Pathways of gastric carcinogenesis, *Helicobacter pylori* virulence and interactions with antioxidant systems, vitamin C and phytochemicals. *International journal of molecular sciences*. 2020 Sep 3;21(17):6451. <https://doi.org/10.3390/ijms21176451>
7. Torbenson M, Ng I, Park Y, Roncalli M, Sakamoto M. WHO Classification of Tumours. Digestive System Tumours. Lyon, France: International Agency for Research on Cancer. 2019:229-39. <https://dokumen.pub/who-classification-of-tumours-5th-edition-digestive-system-tumours-1-5nbsped-9789283244998.html>
8. Lauwers G, Kumarasinghe P, Cytopath D. Gastric cancer: Pathology and molecular pathogenesis. UpToDate, UptoDate, Waltham, MA. 2020. <https://www.medilib.ir/uptodate/show/119517>
9. Nishizawa T, Nakano K, Harada A, Kakiuchi M, Funahashi S-I, Suzuki M, et al. DGC-specific RHOA mutations maintained cancer cell survival and promoted cell migration via ROCK inactivation. *Oncotarget*. 2018;9(33):23198. <https://doi.org/10.18632/oncotarget.25269>
10. Nakayama I, Shinozaki E, Sakata S, Yamamoto N, Fujisaki J, Muramatsu Y, et al. Enrichment of CLDN18-ARHGAP fusion gene in gastric cancers in young adults. *Cancer Science*. 2019;110(4):1352-63. <https://doi.org/10.1111/cas.13967>
11. Al-Badri BA, Ali GQ. P53 Expression in Gastric Dysplasia and carcinoma in Erbil city. *Journal of the Faculty of Medicine Baghdad*. 2011;53(2):190-3. <https://doi.org/10.32007/jfacmedbagdad.532869>
12. Habib M. The possible role of EBV in carcinogenesis of colorectal carcinoma. *Fac Med Baghdad*. 2010;52:172-4. <https://doi.org/10.32007/jfacmedbagdad.5221016>
13. Yang J, Liu Z, Zeng B, Hu G, Gan R. Epstein-Barr virus-associated gastric cancer: a distinct subtype. *Cancer letters*. 2020 Dec 28;495:191-9. <https://doi.org/10.1016/j.canlet.2020.09.019>
14. Ramos MFKP, Pereira MA, de Mello ES, dos Santos Cirqueira C, 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 Journal of Clinical Oncology*. 2021;12(8):688. <https://doi.org/10.5306/wjco.v12.i8.688>
15. Ratti M, Lampis A, Hahne JC, Passalacqua R, Valeri N. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. *Cellular and Molecular Life Sciences*. 2018;75:4151-62. <https://doi.org/10.1007/s00018-018-2906-9>
16. 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*. 2020 Jun 25;12(6):1689. <https://doi.org/10.3390/cancers12061689>
17. 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. *Journal of pathology and translational medicine*. 2020 Jul 1;54(5):378-86. <https://doi.org/10.4132/jptm.2020.06.01>
18. Kim KW, Kim N, Choi Y, Kim WS, Yoon H, Shin CM, et al. Different effects of p53 protein overexpression on the survival of gastric cancer patients according to Lauren histologic classification: a retrospective study. *Gastric Cancer*. 2021 Jul;24:844-57. <https://doi.org/10.1007/s10120-021-01163-y>
19. Zhang X, Wang M, Wang Y, Cheng X, Jiang Y, Xiao H. Clinicopathologic significance of Her-2 and P53 expressions in gastric cancer. *Asian Journal of Surgery*. 2023;46(1):526-31. <https://doi.org/10.1016/j.asjsur.2022.06.039>
20. Karpińska-Kaczmarczyk K, Lewandowska M, Ławniczak M, Bialek A, Urasińska E. Expression of mismatch repair proteins in early and advanced gastric cancer in Poland. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2016;22:2886. <https://doi.org/10.12659/MSM.897150>
21. Hanon BM, Mohammad NAA-M, Mahmood AS. The Correlation Between Microsatellite Instability and the Features of Sporadic Colorectal Cancer in Sample of Iraqi Patients. *Journal: Journal Of Advances In Biotechnology*.4(1). <https://doi.org/110.24297/jbt.v4i1.1632>
22. Tanabe H, Mizukami Y, Takei H, Tamamura N, Omura Y, Kobayashi Y, et al. Clinicopathological characteristics of Epstein-Barr virus and microsatellite instability subtypes of early gastric neoplasms classified by the Japanese and the World Health Organization criteria. *The Journal of Pathology: Clinical Research*. 2021;7(4):397-409. <https://doi.org/10.1002/cjp2.209>
23. Reitsam NG, Märkl B, Dintner S, Waidhauser J, Vlasenko D, Grosser B. Concurrent loss of MLH1, PMS2 and MSH6 immunoexpression in digestive system cancers indicating a widespread dysregulation in DNA repair processes. *Frontiers in Oncology*. 2022;12:1019798. <https://doi.org/10.3389/fonc.2022.1019798>
24. Evaristo G, Katz A, Ramírez-García Luna JL, Issac MS, Sangwan V, Thai D-V, et al. Relation between mismatch repair status, chemoresponse, survival and anatomic location in gastroesophageal adenocarcinoma. *Canadian Journal of Surgery*. 2023;66(1):E79. <https://doi.org/10.1503/cjs.017021>
25. Zhang L, Wang Y, Li Z, Lin D, Liu Y, Zhou L, et al. Clinicopathological features of tumor mutation burden, Epstein-Barr virus infection, microsatellite instability and PD-L1 status in Chinese patients with gastric cancer. *Diagnostic Pathology*. 2021;16(1):38. <https://doi.org/10.1186/s13000-021-01099-y>

26. Elrefaey HA, Mohamed DA, Al-Azim MAA-HA, El-Shorbagy SH, Helal DS. Microsatellite Instability and P53 Aberrant Expression in Gastric Adenocarcinoma: Immunohistochemical Assessment and Correlation with Clinico-pathological Characteristics. *Journal of Advances in Medicine and Medical Research*. 2023;35(14):21-42. <https://doi.org/10.9734/jammr/2023/v35i145052>

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علاقة المعلمات المناعية ل (P53) وعدم استقرار الساتل الميكروي (MSI) مع سرطان المعدة الغدي

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الخلاصة

الخلفية: يحدث سرطان المعدة الغدي بسبب عدم استقرار الكروموسومات، وطفرات TP53، واختلال الصبغة الصبغية، والانتقالات، والجينات الورمية الأولية، والتغيرات الجينية المثبطة للورم. يؤدي عدم استقرار الساتل الميكروي (MSI) إلى فشل إصلاح عدم تطابق الحمض النووي، مما يؤثر على دقة تكرار الحمض النووي. يتم الكشف عن أخطاء النسخ المتمثلة المبكرة بواسطة مجمع البروتين المتغاير (hMSH2/hMSH6)، الذي يقوم بتنشيط (hMLH1 و hPMS2) لإعادة تكوين الحمض النووي. يحدث عدم استقرار الساتل الميكروي في حالات سرطان المعدة المتفرق ومتلازمة لينش.

الهدف من الدراسة: دراسة العلاقة بين تعبير المعلم P53 وتعديل المعلمات المناعية لعدم استقرار الساتل الميكروي (MSI) مع العوامل السريرية المرضية لسرطان المعدة الغدي باستخدام الكيمياء النسيجية المناعية.

المواد والطرق: تم فحص 40 كتلة من نسيج سرطان المعدة الغدي المثبت بالفورمالين والمطموح بالشمع في بغداد، العراق. تناولت الدراسة حالات سرطان المعدة الأولية، مع البيانات السريرية المرضية المتاحة، والأنسجة الجراحية. تم تقييم التعبير المناعي الكيميائي بواسطة نظام تسجيل النقاط. تم استخدام برنامج SPSS لتحليل البيانات، كما تم استخدام اختبارات Chi-square واختبارات Fisher الدقيقة لتقييم الارتباطات. تم اعتبار مستوى الثقة 95% والقيمة الاحتمالية P 0.05 أو أقل مهماً.

النتائج: كان التصبغ المناعي النسيجي الكيميائي لـ P53 إيجابياً في 65% من الحالات، بينما كانت نتائج MSI إيجابية في 97.5% من الحالات. حصل الزوجان المتغايران MLH1/PMS2 على نتائج إيجابية بنسبة 32.5% ونتائج سلبية 67.5%، في حين حصل الزوجان المتغايران MSH2/MSH6 على نتائج إيجابية بنسبة 87.5% ونتائج سلبية 12.5%. ارتبطت صبغة P53 بشكل كبير بانتشار العقدة الليمفاوية ودرجة الورم، ولكن لم يكن هنالك ارتباط مع العوامل الأخرى. لم يتم العثور على ارتباط كبير بين معلمات MSI والعوامل المدروسة ولم يكن هناك ارتباط كبير بين معلمات MSI غير المتجانسة (MLH1 / PMS2) والمعلومات المرضية السريرية، ولكن كان هناك ارتباط كبير بين علامات MSI الزوجين غير المتجانسة (MSH2 / MSH6) والنقائل فقط.

الاستنتاج: يعد P53 معلماً حيوياً مهماً لتقييم انتشار العقدة الليمفاوية وعدوانيتها في التصنيف النسيجي، مما يشير إلى التشخيص، وتحديد مرضى السرطان الأكثر عرضة لخطر الإصابة بالورم النقيلي. على الرغم من أن MSH2/MSH6 أظهر ارتباطاً مهماً مع النقائل الورمية، إلا أن معلمات MSI كان لها أقل قيمة إنذارية في دراستنا. هناك حاجة إلى مزيد من البحوث لإثبات فعاليتها في علاج سرطان المعدة.

الكلمات المفتاحية: سرطان المعدة الغدي، الكيمياء المناعية النسيجية، عدم استقرار الساتل الميكروي، التصنيف الجزيئي، بروتين مثبط الورم 53.