Molecular classification of gastric Adenocarcinoma based on immunohistochemistry

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Abstract

Background: The Cancer Genome Atlas (TCGA) classified 20% of stomach cancers as genetically stable (GS), aneuploidy, and early-identified, with 73% diffuse subtype enrichment and cadherin-1 (CDH1) somatic mutations. Chromosomally unstable (CIN) stomach malignancies are 50% more prevalent in esophago-gastric junction tumors. Microsatellite instability (MSI) results from DNA mismatch repair errors. The MMR system corrects base mismatches, insertions, and deletions for accurate DNA replication. Lynch syndrome raises young people's risk of colorectal, endometrial, ovarian, and stomach cancer due to autosomal dominant MMR gene mutations. Epstein-Barr virus (EBV) causes breast, lung, stomach, colon, and lung cancers. EBV gene expression and host genome control alter stomach cancer cell cycle pathways and gene expression. EBV-positive gastric tumors (9% of stomach cancers) usually affect men. According to the Asian Cancer Research Group (ACRG), gastric cancer has four molecular classifications: microsatellite stability with epithelial-mesenchymal transition (MSS/EMT), MSS/TP53+ aberrant, which commonly has a higher EBV etiology, and MSS/TP53 normal subgroups; and distal stomach microsatellite instability (MSI).

Aim of the study : We examined the potential of immunohistochemistry to molecularly classify gastric adenocarcinoma according to (TCGA) and (ACRG) algorithms and genetic classification.

Material and methods : From Baghdad, Iraq, we gathered 40 formalin-fixed paraffin-embedded stomach cancer tissue blocks. Primary gastric adenocarcinoma cases were studied using available clinicopathological data and surgical tissues. The immunohistochemical expression assessed by scoring systems and referenced algorithms was used to molecularly classify the gastric adenocarcinoma cases.

Results: IHC staining for P53 was positive in 26 cases (65%) and negative in 14 (35%). EBV-Latent membrane protein (LMP) immunological staining was positive in 27 (67.5%) and negative in 13 (32.5%) cases. A score of 0 (negative) was found in 2 cases (5%), score 1 in 6 (15%), score 2 in 12 (30%), and score 3 in 20 (50%) of E-Cadherin IHC staining. MSI was positive in 39 (97.5%) and negative in 1 (2.5%). The MLH1/PMS2 heterodimeric couple had 13 positive and 27 negative cases. The MSH2/MSH6 heterodimeric couple was positive at 87.5% and negative at 12.5%. Based on the suggested algorithm for TCGA Group A, 67.5% (27 cases) of the forty patients tested positive for the Epstein-Barr virus. Twelve MSI cases (30% of the samples) were found. No genomically stable cases were observed, indicating 0% of the cases, and one case was

referred to as chromosomal instability (2.5%). For (ACRG) Group B, 27 MSI cases (67.5% of the sample) were found, one case classified as MSS/EMT (2.5%), and twelve people (30%) had MSS/aberrant P53. MSS/normal P53 cases were not reported.

Conclusion: Immunohistochemical analysis can be used to distinguish immunophenotypic groups in gastric adenocarcinoma, revealing distinct clinicopathological characteristics. This study demonstrates the classification of gastric adenocarcinoma into biologically and clinically distinct subgroups, facilitating clinical diagnosis.

Keywords: gastric adenocarcinoma, molecular classification , immunohistochemistry , P53 , microsatellite instability.

Introduction

Stomach cancer is the fourth leading cause of cancer mortality, with 1.1 million cases and 75% in Asia, according to GLOBOCAN 2020. Five-year survival is 20%, highest in Eastern Asia (22.4 per 100,000). [1,2] Stomach cancer, Iraq's second-leading killer, killed 783,000 individuals and created over 1,000,000 new cases with clinical, genetic, morphological, epidemiological, and developmental problems in 2018. [3] Environmental and genetic factors, especially Helicobacter pylori, make it the fifth most common and third major cause of death worldwide. [4]. GLOBCAN 2021 predicted Iraqi stomach cancer incidence, mortality, and prevalence: New cases of all ages were 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]. Helicobacter pylori, the first bacterial carcinogen, causes most non-cardia stomach cancers. Childhood acquisition of the bacteria can cause diffuse stomach cancer, non-cardia intestinal gastric adenocarcinoma, and gastric B-cell lymphocyte mucosa-associated lymphoid tissue lymphoma.[7] As H. pylori eradication may restore atrophic gastritis but not intestinal metaplasia, focused intervention before stomach precancerous alterations may help high-risk individuals avoid gastric cancer. [8,9.10]. With 45-75% exposure, dietary nitrite-secondary amines cause stomach cancer. Stomach cancer is prevented by vitamin C, onions, garlic, and shallots. [11]. High-temperature, protein-rich diets produce carcinogenic heterocyclic amines. [12, 13]. Soy sauce, salted fish, pickled vegetables, and cured meat could cause stomach cancer in 1959. More salt can irritate and colonize H. pylori. [14-17]. Smoking increases stomach cancer risk due to harmful chemicals, heavy metals, carcinogens, nitrosamines, nicotine, reactive oxygen species, reactive nitrogen species, and lipid peroxidation. [18,19]. Gastric cancer risk is increased by smoking, which reduces prostaglandin synthesis, gastric mucus integrity, glutathione, and vitamin C. [20]. A meta-analysis found that 10 grams of alcohol raises the stomach cancer risk by 7%. Alcohol metabolites increase nitrosamine absorption and non-cardia stomach adenocarcinoma by damaging the stomach mucosa. [21,22]. BRCA2, HNPCC, Lynch II, Li Fraumeni, and FAP syndrome increase stomach cancer risk. [23]. The 2019 WHO classification divides malignant gastric epithelial tumors into tubular, papillary, weakly cohesive signet ring phenotype, poorly cohesive other cell type, mucinous, and mixed histological types. [24]. Intestinal and diffuse gastric cancer subtypes differ in shape, epidemiology, pathogenesis, and genetics. Males and elderly people have a better tubular or glandular intestinal cancer prognosis. Poorly cohesive carcinomas infiltrate glandless tissues, while signet ring cell carcinomas invade surrounding tissues [25–27]. Globally, Epstein-Barr virus stomach cancer rates are 2–20%, averaging 10%. [28–30]. Rare stomach cancers, such as adenosquamous and squamous cell carcinoma,

micropapillary carcinoma, and fundic gland cancer, have a poor prognosis and significant metastatic rates. They rarely enter the lymphatic or venous systems. [31-33]. The Cancer Genome Atlas (TCGA) suggested that 20% of stomach tumors are genetically stable, aneuploid, and early-identified, with 73% diffuse subtype enrichment and cadherin-1 (CDH1) somatic mutations. [27, 34–37]. Chromosomally unstable stomach cancers are 50% more common in esophagogastric tumors. A cell cycle regulator, P53, minimizes DNA replication errors during synthesis, decreasing cancer progression. At 17p, mutation or heterozygosity loss often inactivates it. [38]. Trastuzumab can treat 10–20% of gastric adenocarcinomas if HER2 is overexpressed; thus, intestinal shape, 70% TP53 mutations, and histological P53 overexpression are important genetic factors. [27,39]. DNA mismatch repair difficulties induce microsatellite instability. The Mismatch Repair (MMR) system—hMLH1, hMSH2, hMSH6, and hPMS2 proteins—corrects base mismatches, insertions, and deletions for DNA replication accuracy.[40–42]. Lynch syndrome, caused by autosomal dominant MMR gene mutations, raises young people's colorectal, endometrial, ovarian, and stomach cancer risk. Mutations occur in 22% of microsatellite-unstable gastric tumors. [42-46]. Herpes virus Epstein-Barr causes breast, lung, stomach, colon, and lung malignancies in oropharyngeal B-cells. Cell differentiation and viral gene expression differ in gastrointestinal and nasopharyngeal cancers, complicating cell-environment-viral-mode interactions. [47]. EBV gene expression and host genome control affect stomach cancer cell cycle pathways and gene expression. Recent research links the viral latent profile to latency I or II and latent membrane protein (LMP). EBV-positive gastric tumors (9% of stomach malignancies) mostly affect men. Genetically stable subtypes have the worst prognosis, while EBV-related subtypes do well. [48-57]. Manal A. Habib found EBV DNA in 40 paraffin-embedded colorectal tumors (30 adenocarcinomas and 10 benign) in 2009. Her results indicated in situ hybridization of EBV DNA signals in 20% of colorectal cancer sections but not benign group parts. She found EBV infection in human colorectal adenocarcinoma linked to carcinogenesis [47]. The Asian Cancer Research Group (ACRG) found four molecular categories for gastric cancer. The second algorithm includes the mesenchymal group with microsatellite stability and epithelial-mesenchymal transition (MSS/EMT), which accounts for 15.3% of cases and is usually found in the advanced stages of signet ring cell carcinomas. The second MSS/TP53-negative subtype accounts for 35.7% of cases, while the third has an MSS/TP53+ aberrant subtype, which has higher EBV infection rates. Lastly, the fourth, microsatellite instability (MSI), starts in the distal stomach and has the greatest prognosis [58].

Material and methods

In our retrospective cohort study, we used formalin-fixed, paraffin-embedded tissue blocks from 40 gastric cancer patients. The histopathology department of the Gastroenterology and Hepatology Teaching Hospital, Teaching Laboratory Institute, and some private laboratories in Baghdad, Iraq, had provided us with 2020–2023 archived samples. Data and materials were collected in September 2022–September 2023. All patients underwent a possible curative complete, proximal, and distal gastrectomy with lymphadenectomy and were histologically classified as 19 intestinal, 14 diffuse, and 7 mixed. The cases included 23 males and 17 women aged 24–75. The first of eight 5-micrometer sections from each block was stained with hematoxylin and eosin for histopathological review, while the other seven were stained with immunohistochemistry for immune marker expression, i.e., P53, EBV, E-cadherin, and MSI (MLH1, MSH2, MSH6, PMS2). The tissue processing and staining were done in a private laboratory. The study includes cases with primary gastric adenocarcinoma, available clinicopathological data, and surgical specimens with

available tissue for paraffin blocks. Other gastric tumors, secondary gastric adenocarcinomas, endoscopic biopsies, and gastric cancers with pre-operative neoadjuvant therapy were excluded from the study.

Materials

The materials used in this study were: Xylene Analar (England), absolute ethanol Merck (Germany), distilled water, rinse buffer TBS (DakoCytomation), and 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, primary antibody: DAKO FLEX monoclonal mouse Anti-Epstein-Barr Virus, LMP, Clone CS.1-4(isotype: IgG1, kappa) Ready-to-use (Link) Code IR753, primary antibody: DAKO FLEX monoclonal mouse Anti-Human E-Cadherin, clone NCH-38 (isotype: IgG1, kappa). Ready-to-use (Link), Code IR059, primary antibody DAKO MLH1 Clone ES05 Ready-to-use (Prediluted) Product no./lot number: IR079/IS079/11450820, primary antibody: DAKO MSH2 Clone FE11 Ready-to-use (Prediluted) Product no./lot no.: IR085/10148024, primary antibody: DAKO MSH6 Clone EP49 Ready-to-use (Prediluted) Product no./lot no.: IR086/11166400, primary antibody: DAKO PMS2 Clone EP51 Ready-to-use (Prediluted) Product no./lot no.: IR087/11170264, Hematoxylin Counterstain 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, MHL1, MSH2, MSH6, and PMS2.

Methods

The process involved deparaffinizing the blocks in an oven at 60°C for 1 hour, followed by two 5-minute xylene swaps. The tissues were then rehydrated at decreasing concentrations of ethanol for 5 minutes. Hematoxylin was applied for nuclear staining, followed by hydrochloric acid for differentiation. Eosin was used as a counterstain. Dehydration in absolute alcohol was done for 3-5 minutes, followed by a 5-minute xylene clearing. DPX mounting was then done. H & E slides were examined to choose the best sections for immunohistochemical marker expression. For IHC, the study involved sectioning tissue blocks at 5 microns using a microtome, incubating them in a 45°C water bath, and deparaffinizing them by heating them in a 65°C oven for 30 minutes. Alcohol solutions were used to rehydrate the tissue, and the slides were washed with tap water. Antigen retrieval involved heating a pH 9 tris-ethylene diamine terta-acetic acid (TRIS EDTA) solution in a water bath at 65°C, raising it to 97°C for 20 minutes, and then lowering it back to 65°C. Reagent blockers like PAP pens were used to maintain the agent in the tissue. A wash buffer solution was used, and then two drops of peroxidase blocker were applied to the sample to stop the endogenous antigen activity. Primary antibodies were used to stain the samples, including P53, EBV-LMP, E-CADHERIN, MLH1, MSH2, MSH6, and PMS2. Horseradish peroxidase was applied to the secondary antibody, i.e., anti-mouse-anti-rabbit, and washed twice. Poly detector DAB chromogen was applied to enhance HRP interaction, preparing (3,3-diaminobenzidine) DAB. Three buffer changes were made during a 5-minute wash. Hematoxylin counterstain was applied to the background, dehydrated with alcohol solutions, and DBX was used with a coverslip.

For the interpretation of the immune markers, we stained the tumor cell labeling with monoclonal antibodies for mismatch repair (MMR) markers (MLH1, MSH2, MSH6, and PMS2). Some studies consider gastric cancer negative if tumor cells are unstained for all markers [59]. Others argued that MMRD, an indirect sign of MSI, is supported by the loss of expression of a single protein or a heterodimeric pair of the MMR complex. While hMLH1 and hMSH2 are stable without their dimeric companions, hPMS2 and hMSH6, these components are rarely stable. Gastric cancer with negative MLH1, MLH2, MSH6, and PMS2 expression lacks MMR protein, forming the MSI profile. [60]. Positive internal controls included lymphocytes, fibroblasts, and normal gastric epithelium near the tumor. The expression was lost when nuclear staining was lacking in cancer cells but present in normal gastric epithelium, lymphocytes, and fibroblasts.

E-cadherin, which is usually expressed as membranous and/or cytoplasmic staining, was scored. Scores 0 (total loss) and 1 (cytoplasmic expression) were abnormal, whereas scores 2 (cytoplasmic and membrane labeling) and 3 (membranous) were normal. [59] E-cadherin is expressed in normal tissues such as the urothelium, human mammary gland, esophageal squamous epithelial cells, gastric mucosa, crypts, and deep gastric glands. In normal skin, basal keratinocytes have E-cadherin immunoreactivity on their lateral and upper surfaces at intercellular borders but not on their basal cell surfaces. The suprabasal layers of skin express E-cadherin consistently around cell peripheries, whereas the superficial corneal layer does not. Membrane staining of outer root sheath cells, sebaceous gland acinar germinative cells, and sweat gland cells shows E-cadherin positivity. Normal skin dermis did not express E-cadherin. Abnormal tissue: primary transitional bladder carcinoma, ductal and lobar breast carcinoma, esophageal squamous cell carcinoma, gastric carcinoma, endometriosis, melanocytic nevi, malignant melanoma, prostate cancer, and various metastatic tumors as listed in the antibody leaflet.

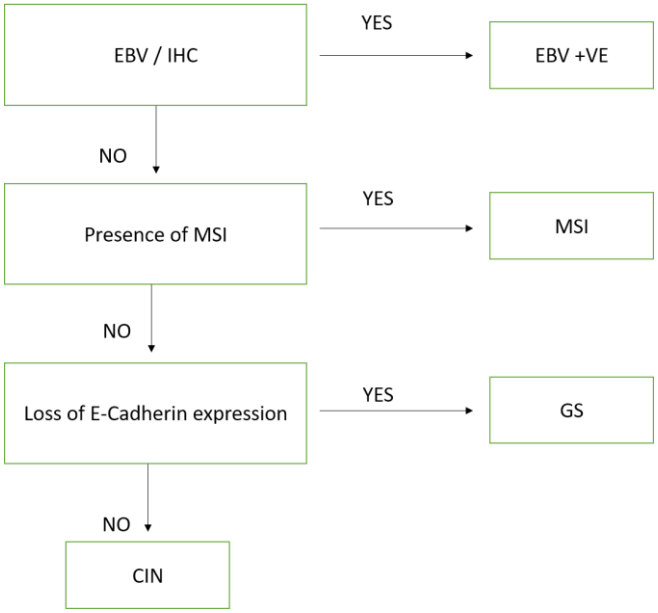
While most antibody-labeled P53 cells stain with nuclear reactions, some have cytoplasmic reactions. Aberrant expression is high nuclear staining in at least 70% of tumor cells and total p53 reaction absence, or less than 5% [59]. The control was benign epithelium and stroma. 27 mesotheliomas have negative cells. Only benign basal epithelial cells in the colon displayed weak to moderate nuclear staining. In follicular lymphoma, centroblasts accumulate more p53. In mesotheliomas, antibodies designate epithelial, mixed, and mesenchymal types, as stated in the antibody leaflet.

The integral membrane protein, LMP-1, is encoded by the BNRF1 gene and contains 386 amino acids with a molecular mass of 63 kDa. It is regarded as an oncogenic protein as it is implicated in at least four transcription factor signaling pathways and induces the expression of multiple cell surface markers and cell adhesion molecules. EBV is associated with certain tumors of lymphoid and epithelial origin, including Burkitt's lymphoma, immunoblastic lymphoma, T-cell lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, and gastric carcinoma (as mentioned in the antibody leaflet). LMP encoded by EBV was used to assess cell EBV status using immunohistochemistry (IHC). IHC is a reliable main screening approach for EBV due to its simplicity, ease, high sensitivity, and low cost, even though it cannot detect the virus's location or transcriptional quantity [59]. In initial nasopharyngeal samples, the antibody does not identify normal mucosa, tiny infiltrating lymphocytes, desmoplastic stroma, or overlaying normal epithelium. The large intestine, appendix, and pancreas are unlabeled. Strong labeling of typical early myeloid and erythroid precursors may be found without EBV confirmation by PCR.

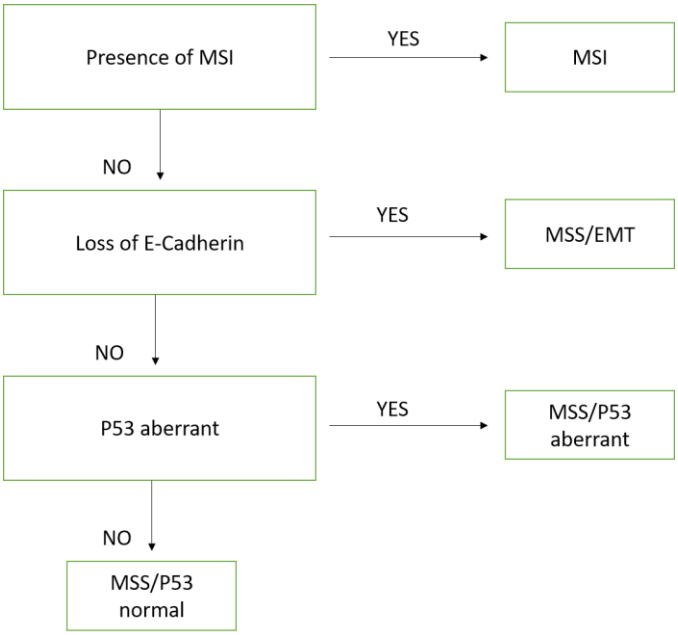
According to the antibody leaflet, the antibody labels Hodgkin's disease, nasopharyngeal carcinomas, and keratinizing and non-keratinizing squamous cell carcinomas.

The cases were classified according to (TCGA AND ACRG) algorithms and genetic classification.[59]

1- Group A
The cancer genomic atlas (TCGA)

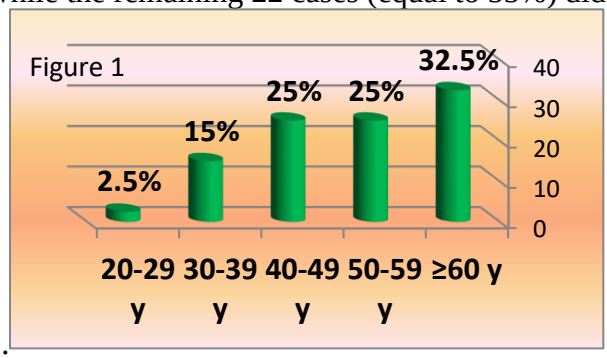


2- Group B
Asian cancer research group (ACRG)



Results

The majority of cases were aged above sixties (Figure 1). The study analyzed 40 gastric adenocarcinoma cases, revealing a gender distribution of 57.5% males and 42.5% females (figure 2), with 10% found in the proximal stomach, 2.5% in the fundus, 50% in the body and antrum, and 37.5% in the distal stomach. Of these cases, 62.5% underwent total gastrectomy, 5% underwent proximal gastrectomy, and 32.5% underwent distal gastrectomy. The cases were categorized based on histopathology reports into 19 cases of intestinal type adenocarcinoma (47.5%), 14 cases of diffuse type adenocarcinoma (35%), and 7 cases of mixed type adenocarcinoma (17.5%), as mentioned in Table 1. The cases were divided into four stages: 1A, 1B, 2A, 2B, 3A, 3B, and Stage 4. The grading of the cases was subdivided into G1 well-differentiated adenocarcinoma, G2 moderately differentiated adenocarcinoma, G2/G3 moderately to poorly differentiated adenocarcinoma, and G3 poorly differentiated adenocarcinoma (table 2). Only 42.5% of the cases showed lymphovascular invasion (17 cases), while 57.5% (23 cases) did not (Figure 3). Perineural invasion was present in 18 cases (equal to 45%), while the remaining 22 cases (equal to 55%) did not show such invasion (Figure 4).



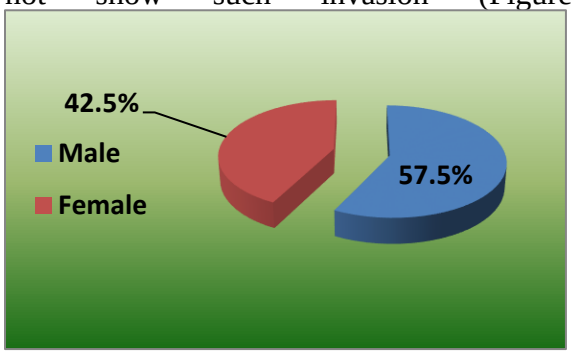
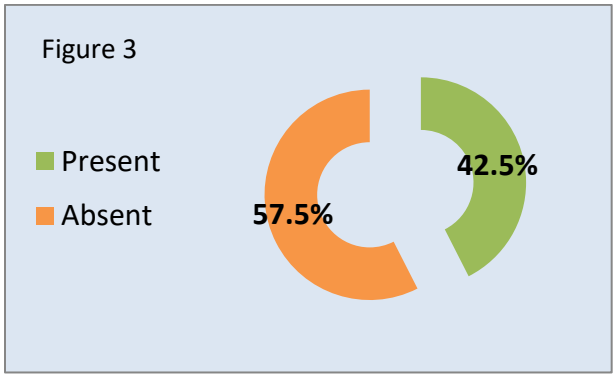


Figure 2

Figure 1: Distribution of participants according to age.

Figure 2: Distribution of participants according to gender.



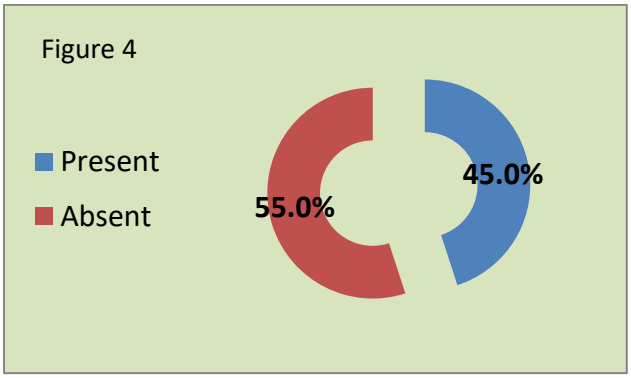


Figure 3: Distribution of participants according to lymphovacular invasion

Figure 4: Distribution of participants according to perineural invasion

Table 1: Site, specimen and diagnosis of the participants, Baghdad,2023.

Variable	Frequency N	Percent %
Site		
Proximal stomach gastroesophageal junction and cardia	4	10
Fundus	1	2.5
Body and antrum	20	50
Distal stomach	15	37.5
Specimen		
Total gastrectomy	25	62.5
Proximal gastrectomy	2	5
Distal gastrectomy	13	32.5
Diagnosis		
Intestinal type adenocrcinoma	19	47.5
Diffuse type adenocarcinoma	14	35
Mixed type adenocarcinoma	7	17.5

Table 2: Stage and grade of the participants, Baghdad, 2023.

Variable	Frequency N	Percent %
Stage		
1A	1	2.5
1B	5	12.5
2A	5	12.5
2B	11	27.5
3A	6	15
3B	10	25
4	2	5
T		
1	2	5
2	7	17.5
3	26	65
4A	4	10
4B	1	2.5
N		
0	11	27.5
1	9	22.5
2	8	20
3	4	10
3A	4	10

3B	2	5
X	2	5
M		
0	1	2.5
1	2	5
X	37	92.5
Grade		
G1 well differentiated	1	2.5
G2 moderately differentiated	24	60
G2/G3 moderately to poorly differentiated	2	5
G3 poorly differentiated	13	32.5

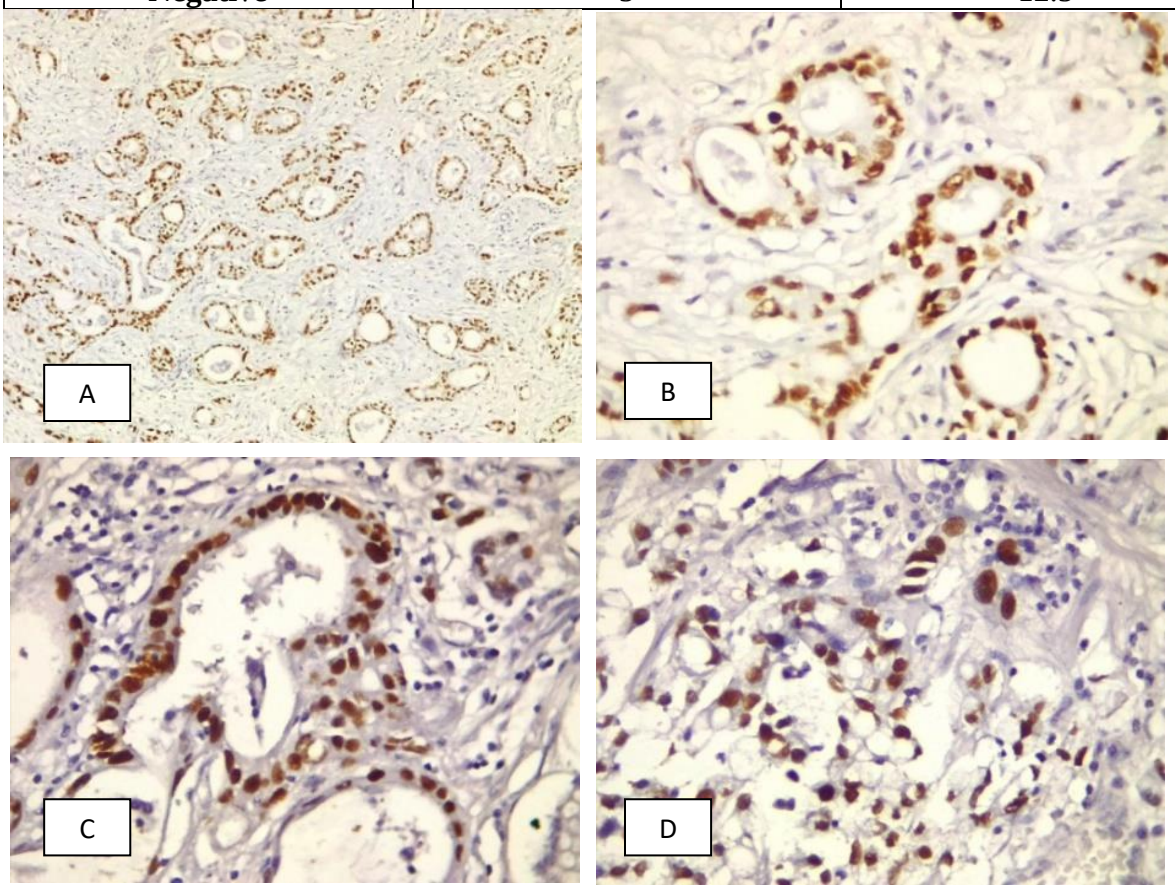
IHC staining for P53 was positive in 26 cases (65%), as shown in picture 1, and negative in 14 (35%). The EBV-LMP immune staining was positive in 27 cases (67.5%), as shown in picture 1, and negative in 13 cases (32.5%). IHC staining for the E-Cadherin scoring system showed Score 0 (negative) for 2 cases (5%), Score 1 (positive cytoplasmic) for 6 cases (15%), Score 2 (positive membranous and cytoplasmic) for 12 cases (30%), and Score 3 (positive membranous) for 20 cases (50%), as shown in Picture 2. A referenced study showed that MSI had positive expression in 39 cases (97.5%), as shown in Picture 3, and negative results in 1 case, as shown in Picture 4 (2.5%). However, another study suggested that MSI immune markers were considered heterodimeric pairs; hence, our investigation found the following: MLH1/PMS2 couples had 32.5% positive results (13 cases) and 67.5% negative results (27 cases). MSH2/MSH6 was positive in 87.5% of cases (35 cases) and negative in 12.5% (5 cases). Table 3 shows the expression of immunohistochemistry markers.

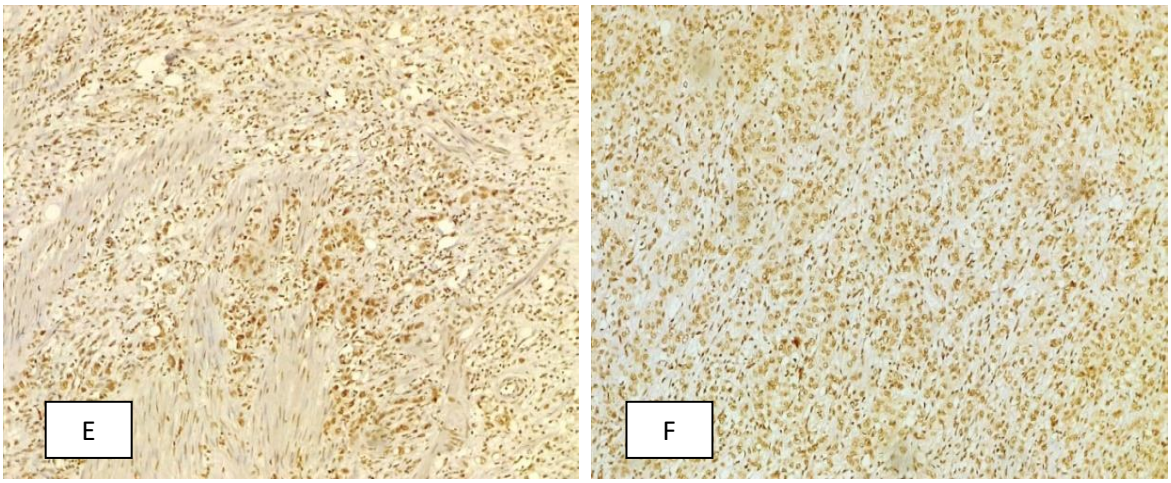
Table 3: Main stains of the participants, Baghdad, 2023.

Variable	Frequency N	Percent %
P53		
Positive	26	65
Negative	14	35
EBV-LMP		
Positive	27	67.5
Negative	13	32.5
E-CADHERIN		
Negative score 0 (abberant expression)	2	5
Positive score 1 cytoplasmic(abberant expression)	6	15
Positive score2 membranous and cytoplasmic	12	30
Positive score 3 membranous	20	50
MSI		
Positive	39	97.5
Negative	1	2.5

Table 4: Stain couples of the participants, Baghdad, 2023

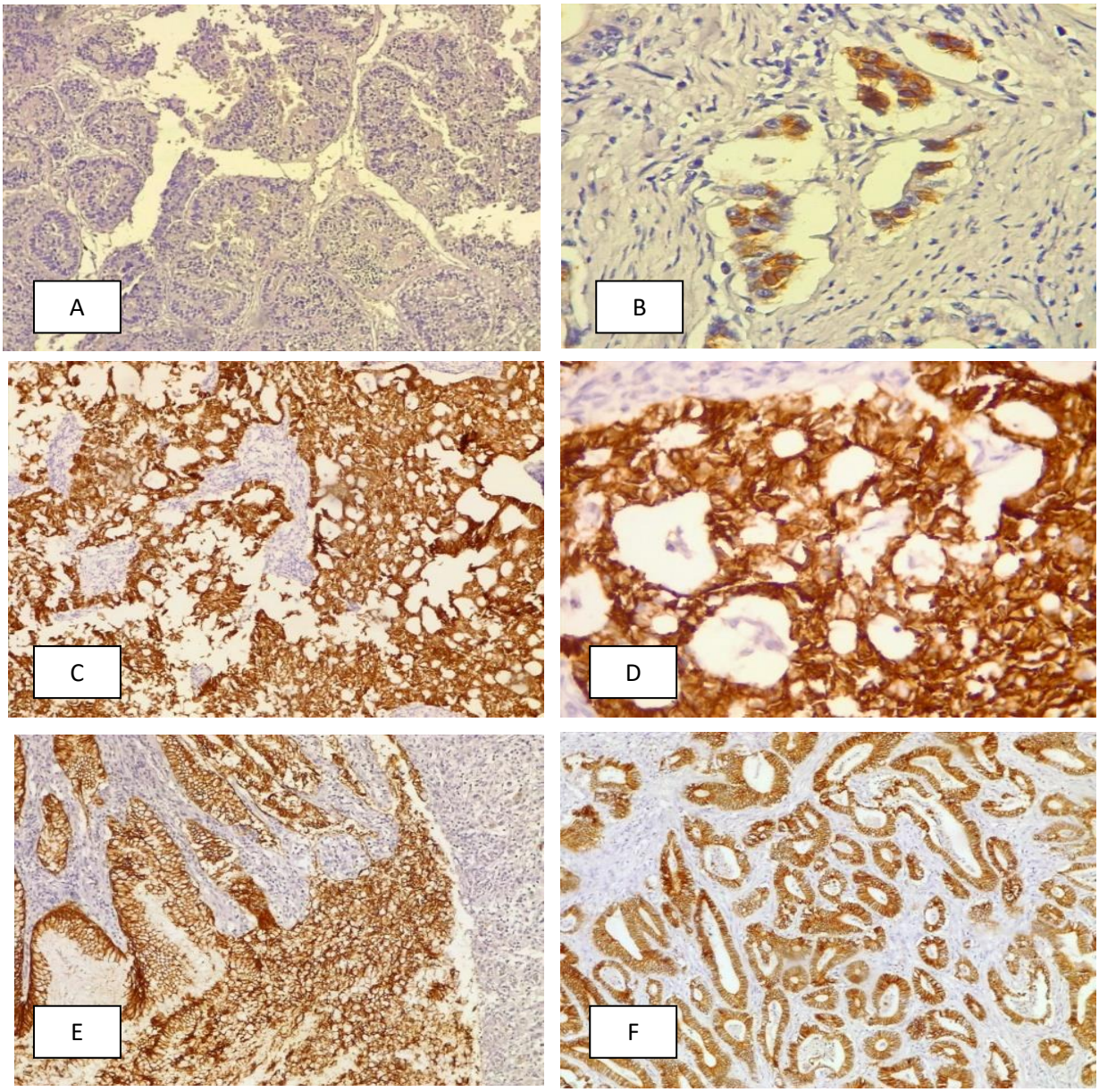
Variable	Frequency N	Percent %
MLH1/PMS2		
Positive	13	32.5
Negative	27	67.5
MSH2/MSH6		
Positive	35	87.5
Negative	5	12.5





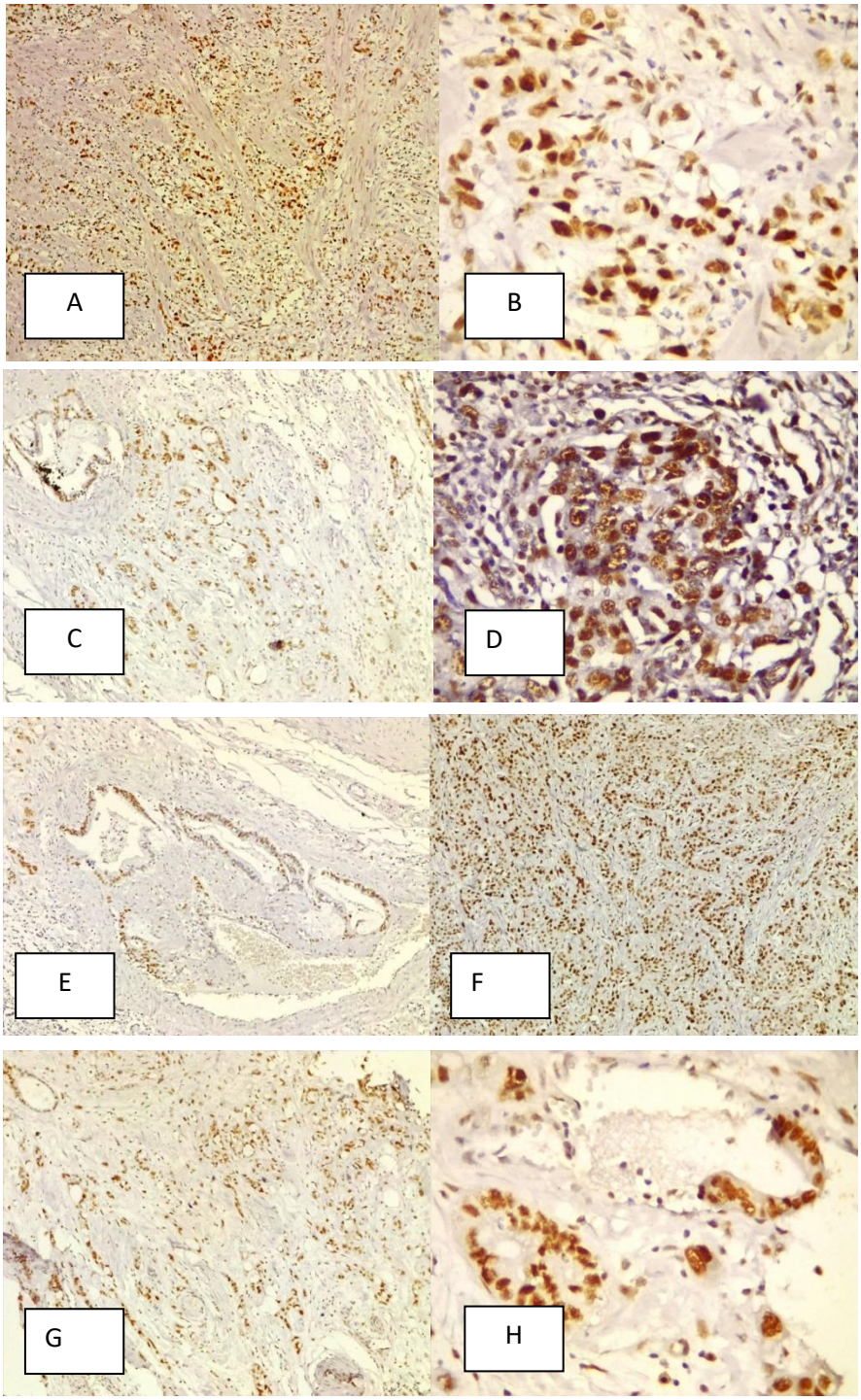
Picture (1) : P53 , EBV-LMP positive immune markers overexpression.

- 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).
- E,F: EBV-LMP positive; 100X



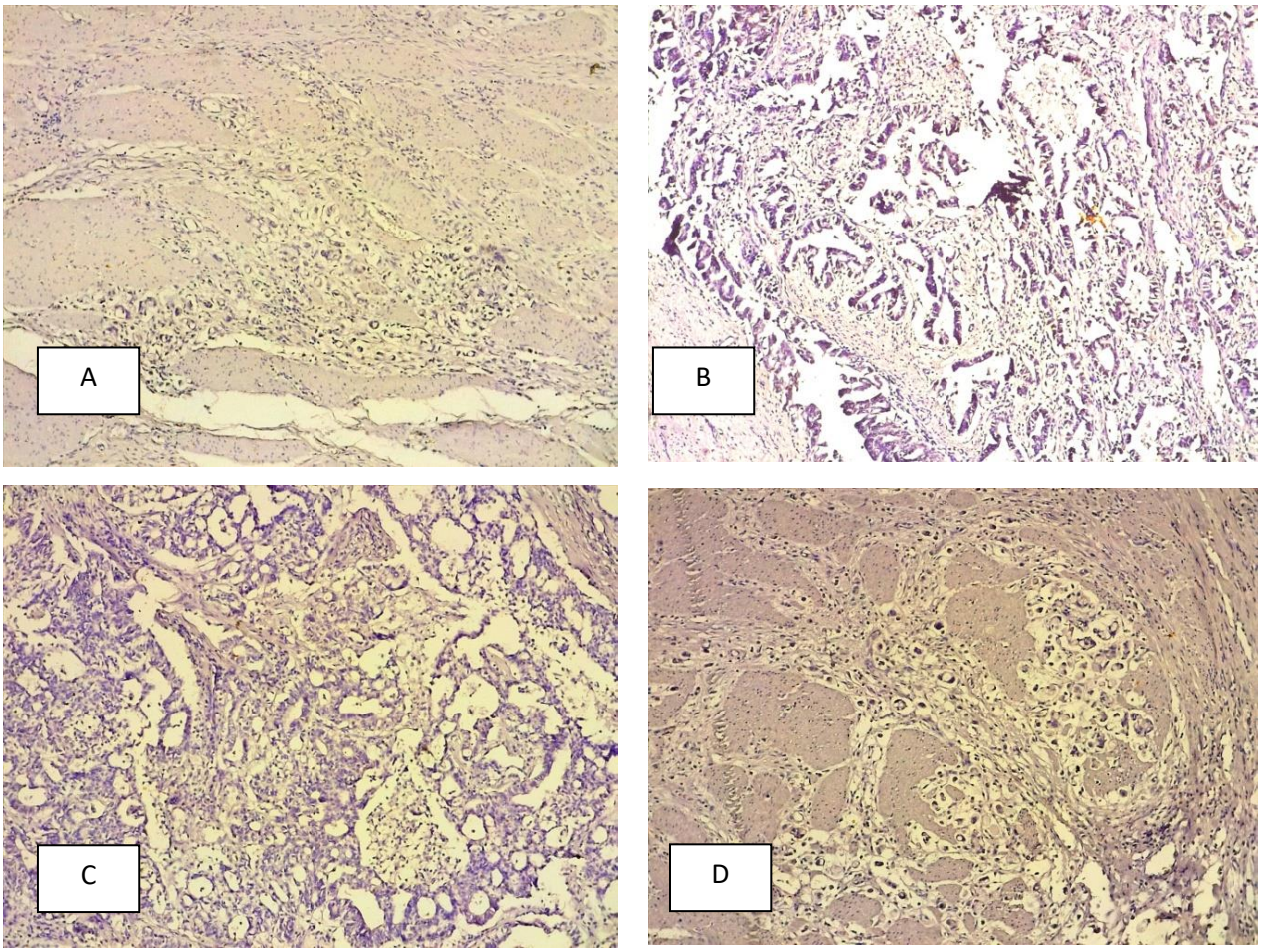
Picture (2) : E-Cadherin immune marker expression.

- A: E-CAD score 0 negative aberrant expression (100x) ,
- B : E-CAD score1 positive cytoplasmic aberrant expression (400x).
- C: E-CAD score 2 positive cytoplasmic and membranous normal expression(100x),
- D: E-CAD score 2 positive cytoplasmic and membranous normal expression(400x).
- E: E-CAD score3 positive membranous normal expression (100x),
- F :E-CAD score 3 positive membranous normal expression (400x).



picture (3) : 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



picture(4): MSI immune markers negative expression.

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

Molecular classification of gastric adenocarcinoma based on immunohistochemistry:

Based on the algorithm proposed, the subsequent outcomes were identified:

1. The Cancer Genomic Atlas (TCGA) Group A: Among the sample of forty patients under our investigation, a total of twenty-seven cases, or 67.5% of the sample, were identified as positive for (EBV-LMP). A total of twelve cases, including thirty percent of the overall sample, were classified as MSI cases. No cases of GS were reported, indicating a 0% incidence rate. In contrast, the frequency of CIN cases was determined to be 2.5%, with only one case reported.
2. The Asian Cancer Research Group Classification (ACRG) Group B: Among the forty cases under our investigation, a total of 27 cases, or 67.5% of the overall sample size, were identified as MSI cases. The prevalence of MSS/EMT cases was determined to be 2.5%, with a singular case being identified. Twelve individuals, including 30% of the sample,

exhibited MSS/aberrant P53. Finally, the prevalence of MSS/normal P53 occurrence was determined to be 0%, with no documented cases observed.

Discussion

The study investigated forty cases, and according to (TCGA) classification, 67.5% of them were positive for (EBV-LMP). A total of twelve cases, including 30% of the overall count, were identified as cases exhibiting microsatellite instability (MSI). No cases of GS were reported. The frequency of CIN cases was determined to be 2.5%, with a single documented case. For the (ACRG) algorithm, a total of 27 cases, or 67.5% of the overall sample size, were identified as MSI cases. The prevalence of MSS/EMT cases was 2.5%, with one solitary case. A number of 12 patients, constituting 30% of the participants, exhibited MSS/aberrant P53. The prevalence of MSS/normal P53 subjects was 0%, with no documented cases. In 2016, R. S. Gonzalez et al. found the following patient groups: group 1 Epstein-Barr encoding region (EBER) positive, 7%; group 2 (MLH1 deficient, 16%); group 3 (aberrant p53 staining, EBER negative, retained MLH1), 40 (38%); and group 4 (unremarkable). After controlling for chromosomal instability's TP53 mutation rate, the Research Network found a similar pattern. Patients in Group 1 had longer follow-ups (median, 70 months vs. 13 months for other groups; P =.0324). None of the group 2 cases overexpressed HER2. Group 3 has 3 HER2 immunohistochemistry-positive patients and 7 fluorescence-in situ hybridization (ISH)-positive patients. [61] Ramos MFKP et al. analyzed 287 gastric cancer patients in 2021. The IHC and ISH revealed five profiles. These profiles were E-cadherin aberrant (9.1%), MSI (20.9%), p53 aberrant (36.6%), EBV positive (10.5%), and p53 normal (31%). Importantly, tumors with one profile did not change the others. A flowchart based on TCGA and ACRG classifications defines gastric cancer subgroups. This method identified subtype-specific clinical and pathological criteria. [59] In 2022, Valentina Angerilli et al. classified dysplastic lesions as follows: TCGA classification: EBV, 0/73 (0%), MSI, 6/73 (8.2%), GS, 4/73 (5.5%), CIN, 63/73 (86.3%); ACRG molecular subtyping: MSI, 6/73 (8.2%), MSS/EMT, 4/73 (5.5%), MSS/TP53−, 33/73 (45.2%), MSS/TP53+, 30/73 (41.1%) [62]. Our molecular classification results for gastric adenocarcinoma disagree with the previous results of the studies mentioned above because we used immunohistochemical expression of EBV-LMP to assess the EBV status, which may have affected the classification algorithm, while they used ISH for EBV detection, which was more specific.

Conclusion

The immunohistochemical analysis could be used to distinguish the immunophenotypic groups of gastric adenocarcinoma with distinct clinicopathological characteristics. Therefore, this study shows that gastric adenocarcinoma can be classified into biologically and clinically different subgroups by using simple methods that are also applicable for clinical diagnosis.

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Authors' declaration:

There was no conflicts of interest. All of the figures and tables in the text are original works by myself or my collaborators. Authors sign off based on their acceptance of ethical considerations. Meeting Ethical Requirements: Ghazi Al-Hariri Hospital's local ethics committee in Medical City gave their stamp of approval to the study, according to the code no.145(4THOCT-2022).

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