

Prevalence and co-detection of *Helicobacter pylori*, Epstein–Barr virus, and human papillomavirus in gastric tissues: a cross-sectional study from Yazd, Iran (2019–2022)

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ABSTRACT

Background and Objectives: Gastric cancer remains a leading cause of cancer mortality and exhibits significant geographic variation. Although *Helicobacter pylori* (*H. pylori*) is a well-established carcinogen, the contributions of Epstein–Barr virus (EBV) and human papillomavirus (HPV), individually or in conjunction with *H. pylori*, are not fully understood. This study assessed the prevalence and co-detection of these pathogens in gastric tissues from Yazd, Iran.

Materials and Methods: An archive-based cross-sectional study was conducted using 130 formalin-fixed, paraffin-embedded (FFPE) gastric specimens collected between 2019 and 2022, comprising 83 gastric adenocarcinomas and 47 non-malignant tissues. *H. pylori* was detected by conventional PCR targeting 16S rRNA, while EBV and HPV were identified by nested PCR targeting *EBNA-1* and *L1*, respectively. PCR products were confirmed by agarose gel electrophoresis, and selected amplicons were verified by Sanger sequencing.

Results: *H. pylori* was detected in 74 of 130 specimens (56.9%), with a prevalence of 62.7% in malignant tissues and 46.8% in non-malignant tissues ($p=0.080$). EBV was identified in three malignant cases (3.6%) and HPV in one malignant case (1.2%), with no positive findings in non-malignant samples ($p=0.187$ and $p=0.450$, respectively). Three co-detections were observed in malignant tissues, including two cases of *H. pylori* with EBV and one case of *H. pylori* with HPV. Due to the limited number of EBV- and HPV-positive cases, association analyses lacked statistical power.

Conclusion: *H. pylori* was the predominant microorganism detected, while EBV and HPV were rare and co-detections were infrequent. Larger prospective studies employing complementary molecular techniques and *H. pylori* virulence profiling are required to elucidate the clinical significance of these infections in gastric carcinogenesis.

Keywords: *Helicobacter pylori*; Papillomavirus infections; Epstein-Barr virus infections; Coinfection

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INTRODUCTION

Gastric cancer remains a major cause of cancer death worldwide, but its burden is unevenly distributed. Some regions carry a much heavier load than others, reflecting differences in environment, lifestyle, and infectious exposures (1, 2). Iran follows this pattern as well; risk is higher in the north and northwest than in other provinces, a gradient that has been noted repeatedly in national reports (3). Even with broader access to endoscopy and efforts to eradicate *Helicobacter pylori*, late presentation and limited, practical risk-stratification tools still hinder prevention and early detection (4).

The role of *H. pylori* in gastric carcinogenesis is clear. The organism is a WHO/IARC Group 1 carcinogen and remains the dominant infectious driver of non-cardia gastric cancer (5). Mechanistically, chronic inflammation and strain-specific virulence factors—most notably *cagA* and *vacA*—promote epithelial injury, genomic stress, and oncogenic signaling in the gastric mucosa (6). In parallel, Epstein–Barr virus (EBV) defines a distinct molecular route to disease. EBV-positive gastric cancer is now recognized as a reproducible subtype characterized by Epstein–Barr virus-encoded small RNAs (EBER) positivity within tumor cells, widespread DNA hypermethylation, frequent PIK3CA mutations, and an immune-rich tumor microenvironment (7). These features are clinically significant as they indicate distinct tumor biology and have the potential to inform future therapeutic strategies.

In contrast, the role of human papillomavirus (HPV) in gastric pathology remains unresolved. Although several studies have identified high-risk HPV DNA in gastric lesions, findings differ substantially depending on methodology and study population. Furthermore, a causal relationship analogous to that established in cervical or oropharyngeal cancers has not been demonstrated (8).

Beyond single-pathogen effects, there is growing interest in co-detection—and possible co-infection—of *H. pylori*, EBV, and HPV in the same tissues. Biologically, it is plausible that chronic *H. pylori*-driven injury could facilitate viral colonization, or that viral immune modulation could reshape the bacterial niche. Several reports suggest enrichment of *H. pylori* in EBV-positive tumors and premalignant lesions, and exploratory analyses have linked *cagA*-positive strains to more aggressive behavior

when EBV is also present (9, 10). Still, findings are inconsistent across settings and often limited by small sample sizes, heterogeneous assays, and minimal adjustment for confounders.

In Iran, contemporary studies that evaluate all three agents—*H. pylori*, EBV, and HPV—in the same gastric specimens, and that explicitly report co-detection patterns across malignant and non-malignant tissues, remain scarce. This gap complicates pathogen-aware risk stratification and limits how we interpret PCR-based surveys on Formalin-fixed, paraffin-embedded (FFPE) material.

Accordingly, we performed an archive-based, cross-sectional study of FFPE gastric tissues from Yazd (2019–2022) to estimate the prevalence of *H. pylori*, EBV, and HPV across malignant and non-malignant specimens and to characterize co-detection patterns. Because pathogen detection was based exclusively on PCR assays performed on FFPE tissues, the study was designed to assess pathogen DNA prevalence and co-detection patterns rather than to establish causality or demonstrate biologically active infection.

MATERIALS AND METHODS

Sample collection and study population: formalin-fixed, paraffin-embedded (FFPE). Gastric tissue samples were collected from February 2019 to November 2022 from the archives of the Pathology Department at the Shahid Sadoughi Hospital in Yazd, Iran. Tissue specimens and demographic data were obtained from the pathology database. In this study, 130 specimens were included: malignant (gastric adenocarcinoma) and non-malignant (normal adjacent mucosa ≥ 2 cm from the tumor and/or benign gastritis/hyperplasia).

Data analysis. The collected data and results underwent a thorough analysis using SPSS version 21, ensuring a robust statistical examination and the reliability of the findings. Categorical variables were compared using Fisher's exact test because of sparse cell counts and the low frequency of EBV- and HPV-positive cases. Continuous variables were analyzed using appropriate parametric or nonparametric tests based on the data distribution. Statistical analyses were performed using SPSS version 21, and a p -value < 0.05 was considered statistically significant.

The outcomes of these analyses were systematically organized and presented in tabular form, allowing for clear and accessible interpretation of the data.

Ethical considerations. The samples were collected from existing paraffin-embedded tissues archived in the pathology department, and individual confidentiality and ethical considerations were maintained. The present study was registered with the code IR.SSu.MEDICINE.REC.1401.018 of the ethics committee.

Quantitative and qualitative evaluation of the extracted DNA. Deparaffinization and DNA extraction were performed using the DNeasy FFPE Tissue Kit (ROJE Technologies, IRAN), designed to extract DNA from paraffin-embedded tissues. All the extraction steps were performed according to the manufacturer's instructions. For quantitative evaluation, 2 μ L of the extracted DNA was loaded and analyzed using a NanoDrop spectrophotometer (Epoch Microplate Reader, Bio-Tek, USA). For qualitative evaluation, the presence of the human β -globin as a housekeeping gene was investigated by PCR. Samples in which the presence of this gene was confirmed were considered suitable for subsequent molecular investigation.

β -globin gene amplification. The conventional PCR method was used to amplify the β -globin gene in a 25 μ L reaction volume with the primers listed in Table 1. PCR Product Verification was performed using Gel Electrophoresis on a 1.5% agarose gel with a 50 bp DNA ladder.

Identification of HPV, EBV, and *H. pylori*-specific genes. Nested-PCR for detecting HPV and EBV genomes, along with conventional PCR for detecting *H. pylori*, was conducted using primers (11, 12) detailed in Table 1.

For HPV, both the first and second rounds began with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles comprising denaturation at 94°C, primer annealing, and extension at 72°C. The extension duration was 45 seconds for the first round, 30 seconds for the second round, and 72°C for 7 minutes in the final extension. For EBV, both rounds were initiated with denaturation at 94°C, lasting 10 minutes in the first round and 5 minutes in the second.

This was followed by 35 cycles of denaturation at 94°C for 45 seconds, primer annealing, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 7 minutes. Lastly, *H. pylori* detection involved an initial denaturation at 95°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 50 seconds, primer annealing, and extension at 72°C for 1 minute, finishing with a final extension at 72°C for 10 minutes.

Electrophoresis and sequencing. Agarose gel electrophoresis was employed to analyze the PCR results. Positive controls for each target gene were submitted for sequencing, while samples that displayed the desired bands were randomly selected and sent for sequencing. PCR products were sequenced using the Sanger method. Raw sequence chromatograms were inspected using Chromas software, and the obtained sequences were compared with reference sequences using the NCBI BLAST database.

Table 1. Primers for specific genes noted in this study

Organism/Gene	Fragment Size (bp)	Primer Sequence	Annealing/Time
Human/ β -globin	110	F: ACACAACGTGTTCCTACTAGC R: CTTTCATCCACGTTACCCCAA	50°C/45sec
HPV/L1	450	F1: CGTCCMARRGGAWACTGATC R1: GCMCAGGGWCATAAAYAATGG	50°C/45sec
	150	F2: TTTGTTACTGTGGTAGATACTAC R2: GAAAAATAAACTGTAAATCATATTC	50°C/30sec
EBV /EBNA-1	609	F1: GTAGAAGGCCATTTTCCAC R1: CTCCATCGTCAAAGCTGCA	53°C/45sec
	308	F2: AGATGACCCAGGAGAAGGCCCAAGC R2: CAAAGGGGAGACGACTCAATGGTGT	60°C/45sec
<i>H. pylori</i> / 16srRNA	139	F: GCGACCTGCTGGAACATTAC R: CGTTAGCTGCATTACTGGAGA	57°C/45sec

RESULTS

Sample information. This cross-sectional descriptive study, conducted between February 2019 and November 2022, analyzed 130 paraffin-embedded gastric tissue samples. The study population included 83 samples from malignant tumors (with a mean age of 60.06 years) and 47 samples from benign tumors (with a mean age of 57.94 years). The gender distribution and categorization of sample types for the entire cohort are presented in Table 2. Non-malignant specimens comprised normal adjacent mucosa (≥ 2 cm from tumor) and/or benign gastritis/hyperplasia; grade and stage annotations were available for 72/83 malignant cases due to archival limitations.

DNA quality assessment using β -globin PCR. DNA quality was assessed by PCR amplification of the β -globin gene. Only samples showing the expected 110-bp amplicon were included in subsequent analyses. Samples that remained negative after DNA re-extraction were excluded (Fig. 1).

PCR detection of HPV and EBV in extracted genomic DNA. The detection of 150 bp fragments during the second round of Nested-PCR with specific

L1 primers confirmed the presence of the HPV genome, as shown in Fig. 2. Likewise, the identification of 308 bp fragments using specific EBNA-1 primers in the second round of Nested-PCR verified the presence of the EBV genome, as shown in Fig. 3.

Detection of *H. pylori* using the 16S rRNA gene. The identification of 139 bp fragments using specific 16SrRNA primers indicated the presence of *H. pylori*. This is illustrated in Fig. 4, which shows the agarose gel electrophoresis results for the presence of the 16SrRNA gene. Standard positive and negative controls were included in every PCR run to verify assay specificity and minimize the risk of non-specific amplification.

Table 2. Sample's distribution by sample type and gender.

Sample Type/Gender	Frequency	Percentage
Malignant (Tumor)	83	63.8%
Benign (Non-Tumor)	47	36.2%
Total	130	100%
Male	78	60.0%
Female	52	40.0%
Total	130	100%

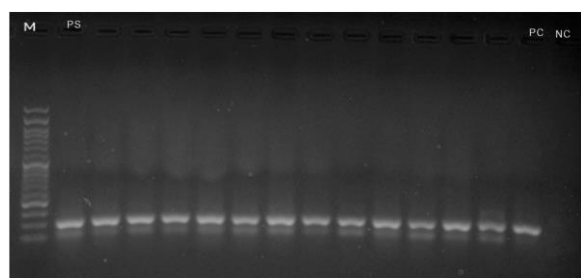


Fig. 1. Representative gel electrophoresis showing amplification of the β -globin gene in samples. 1: M, 50 bp DNA ladder; 2-14: PS: Positive Sample for β -globin gene (110 bp); 15: PC, Positive Control; 16: NC, Negative Control.

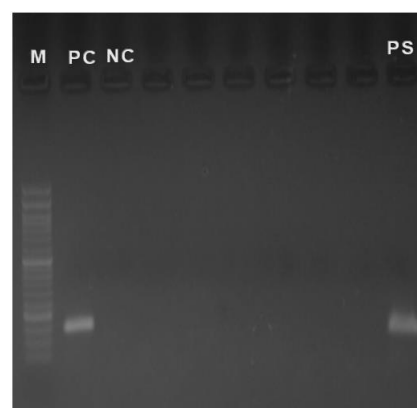


Fig. 2. Agarose gel electrophoresis of PCR products for HPV detection by amplification of the L1 gene. 1: M, 50 bp DNA ladder; 2: PC, Positive Control; 3: NC, Negative Control; 10, PS: Positive Sample for L1 gene (150 bp).

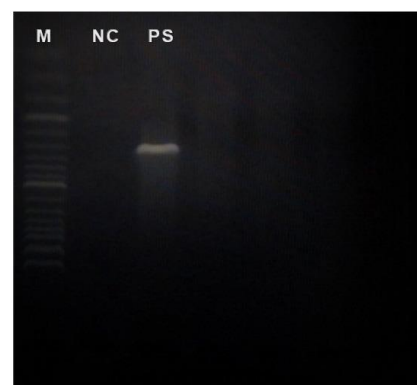


Fig. 3. Agarose gel electrophoresis of PCR products for EBV detection by amplification of the EBNA-1 gene. 1: M, 50 bp DNA ladder; 2: NC, Negative Control; 3: SP, Positive Sample for EBNA-1 gene (308 bp).



Fig. 4. Agarose Gel Electrophoresis of *H. pylori* 16S rRNA Gene Detection. 1: M, DNA ladder 50 bp; 2: NC, Negative Control; 3: PC, Positive Control; 5: SP, Positive Sample for 16S rRNA gene (139 bp).

Overall prevalence of pathogens by sample type (tumor and non-tumor). The relationship between sample type and the presence of the pathogens under study was evaluated. Notably, no concurrent infections involving both microorganisms were observed among the non-tumor samples. Concurrent detections occurred in 3/83 malignant and 0/47 non-malignant samples; given sparse counts, no statistical association was inferred (Fisher's exact; exact 95% CIs reported in Table 3). Additionally, Table 3 presents the relationships between sex and the presence of the studied pathogens. The p-values indicate no statistically significant association between sex and the presence of the pathogens under investigation (Table 3).

Age, grade, and stage analyses. The very small numbers of EBV-positive ($n = 3$) and HPV-positive ($n = 1$) cases substantially limited the statistical power to detect associations with age, tumor grade, and stage. Consequently, the lack of statistically significant findings should not be interpreted as evidence of the absence of an association (Tables 4 and 5).

DISCUSSION

In this archival cross-sectional study of 130 FFPE gastric tissues from Yazd, *H. pylori* was the most frequently detected microorganism, while EBV and HPV were infrequently identified. *H. pylori* was observed more often in malignant than in non-malignant tissues, although this difference did not reach statistical significance. EBV and HPV were detected exclusively in malignant specimens, and co-detections were rare. These results should be interpreted as evidence of the presence of pathogen DNA rather than of biologically active infection or causality in gastric carcinogenesis.

Several methodological limitations should be considered when interpreting these findings in the context of previous studies. Detection relied exclusively on PCR analysis of FFPE tissues, which does not distinguish active infection from latent or incidental pathogen DNA and cannot localize viral genomes within tumor cells. Furthermore, the small number of EBV- and HPV-positive cases limited statistical power and may have obscured weak associations with clinicopathological characteristics. The results reinforce *H. pylori*'s predominance as the principal infectious agent identified in gastric tissues. The prevalence observed in this study (56.9%) exceeds the estimated global prevalence of approximately 44%, yet remains below the 70-90% prevalence reported in many developing countries. This observation aligns with previous research. relatively high burden of *H. pylori* infection reported in Iran and other developing regions further supports its central role in gastric carcinogenesis, despite regional variation in prevalence (4, 5, 13). Reported frequencies vary according to study populations, specimen types, and molecular detection methods (10, 13). Although no statistically

Table 3. Prevalence of *H. pylori*, EBV, and HPV in all samples based on tumor or non-tumor status and gender

Sample Type	<i>H. pylori</i> Positive	<i>H. pylori</i> Negative	Total	EBV Positive	EBV Negative	Total	HPV Positive	HPV Negative	Total
Tumor	52 (62.7%)	31 (37.3%)	83	3 (3.6%)	80 (96.4%)	83	1 (1.2%)	82 (98.8%)	83
Non-Tumor	22 (46.8%)	25 (53.2%)	47	0 (0.0%)	47 (100.0%)	47	0 (0.0%)	47 (100.0%)	47
Total	74 (56.9%)	56 (43.1%)	130	3 (2.3%)	127 (97.7%)	130	1 (0.8%)	129 (99.2%)	130
p-value		0.080			0.187			0.450	
Female	29 (55.8%)	23 (44.2%)	52	0 (0.0%)	52 (100.0%)	52	1 (1.9%)	51 (98.1%)	52
Male	45 (57.7%)	33 (42.3%)	78	3 (3.8%)	75 (96.2%)	78	0 (0.0%)	78 (100.0%)	78
Total	74 (56.9%)	56 (43.1%)	130	3 (2.3%)	127 (97.7%)	130	1 (0.8%)	129 (99.2%)	130
p-value		0.828			0.152			0.219	

Table 4. The relationship between age and the prevalence

	<i>H. pylori</i> Positive	<i>H. pylori</i> Negative	EBV Positive	EBV Negative	HPV Positive	HPV Negative
Count	74	56	3	127	1	129
Mean Age	58.88	84	33.54	41.59	55.00	33.59
p-value	0.756		0.619		0.805	

Table 5. The relationship between cancer grade and pathogen prevalence

Cancer Grade	<i>H. pylori</i> Positive	<i>H. pylori</i> Negative	Total	EBV Positive	EBV Negative	Total	HPV Positive	HPV Negative	Total
G1	8 (72.7%)	3 (27.3%)	11	0 (0.0%)	11 (100.0%)	11	0 (0.0%)	11 (100.0%)	11
G2	14 (70.0%)	6 (30.0%)	20	0 (0.0%)	20 (100.0%)	20	0 (0.0%)	20 (100.0%)	20
G3	22 (59.5%)	15 (40.5%)	37	3 (8.1%)	34 (91.9%)	37	1 (2.7%)	36 (97.3%)	37
G4	2 (50.0%)	2 (50.0%)	4	0 (0.0%)	4 (100.0%)	4	0 (0.0%)	4 (100.0%)	4
Total	46 (63.9%)	26 (36.1%)	72	3 (2.4%)	69 (95.8%)	72	1 (1.4%)	71 (98.6%)	72
p-value	0.718			0.398			0.874		

significant difference was observed between malignant and non-malignant tissues, the established role of *H. pylori* as a gastric carcinogen underscores its importance in gastric carcinogenesis (4, 14).

EBV DNA was detected in only 3.6% of malignant specimens in the present study, which is lower than the approximately 9-10% prevalence of EBV-positive gastric cancer reported worldwide (15). Geographic variation, differences in patient selection, and methodological heterogeneity, particularly the use of PCR instead of EBER in situ hybridization, may explain these discrepancies (7, 16). Because EBV-positive gastric cancer represents a distinct molecular subtype with unique genomic and epigenetic characteristics, confirmation using EBER-ISH is recommended in future studies (7, 15, 16).

HPV was identified in only one malignant specimen and was not detected in non-malignant tissues. This finding aligns with previous studies that report minimal or no evidence supporting a significant role for HPV in gastric cancer (14, 17). Furthermore, meta-analyses have revealed considerable heterogeneity in HPV prevalence among gastric cancer studies, with reported frequencies differing substantially according to geographic region, specimen type, and molecular detection methods (8, 14). Similarly, previous Iranian studies by Fakhraei et al. and Jafari-Sales et al. reported relatively low HPV prevalence in gastric tissues, consistent with our findings (18, 19). Therefore, the detection of isolated HPV

DNA requires careful interpretation.

Although experimental studies have suggested possible interactions between *H. pylori* and oncogenic viruses during gastric carcinogenesis, the evidence remains inconsistent (20, 21). In this study, co-detections were uncommon, and no statistically significant associations were identified. Therefore, these findings neither support nor exclude synergistic interactions among these pathogens, underscoring the need for larger studies with integrated microbial and host molecular analyses.

The strengths of this study include the simultaneous assessment of *H. pylori*, EBV, and HPV in the same FFPE gastric specimens and the evaluation of co-detection patterns in both malignant and non-malignant tissues. Several limitations must be acknowledged when interpreting these findings. These limitations include the cross-sectional study design, the small number of EBV- and HPV-positive cases, the heterogeneous composition of the non-malignant group (including both normal adjacent mucosa and benign gastritis or hyperplasia specimens), the lack of *H. pylori* virulence factor analysis (*cagA* and *vacA*), and incomplete clinicopathological data. Due to insufficient sample sizes within each non-malignant subgroup for reliable statistical analysis, separate pathogen-prevalence analyses were not conducted. Consequently, comparisons between malignant and non-malignant tissues should be interpreted with caution (7, 17, 19, 22).

From a clinical perspective, these findings reinforce the importance of continued *H. pylori* detection and eradication strategies in high-risk populations in Iran (4, 14). In contrast, the low prevalence of EBV and HPV suggests that routine molecular testing for these viruses in unselected gastric specimens is unlikely to provide clinically meaningful information. However, targeted evaluation of EBV using EBER-ISH may be appropriate in selected patients due to the distinct biological and potential therapeutic implications of EBV-positive gastric cancer (15, 16).

Future multicenter studies in Iran should incorporate larger prospective cohorts, EBER-ISH for EBV localization, quantitative HPV assays targeting E6/E7, characterization of *H. pylori* virulence factors (*cagA* and *vacA*), and comprehensive clinicopathological data to clarify the role of pathogen co-detection in gastric carcinogenesis (7, 18). Accordingly, these findings should be regarded as hypothesis-generating and require confirmation in larger prospective studies using complementary molecular techniques.

CONCLUSION

In conclusion, *H. pylori* was the predominant pathogen detected in gastric tissues from this Yazd cohort, whereas EBV and HPV were identified only rarely, and co-detection was uncommon. Although the findings are limited by the cross-sectional design, PCR-based detection, and the small number of EBV- and HPV-positive cases, they provide valuable regional data on pathogen prevalence in gastric tissues. Larger multicenter studies in Iran using complementary molecular and histopathological techniques are warranted further to clarify the role of these pathogens in gastric carcinogenesis.

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REFERENCES

1. Duan Y, Xu Y, Dou Y, Xu D. *Helicobacter pylori* and gastric cancer: mechanisms and new perspectives. *J Hematol Oncol* 2025; 18: 10.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; 68: 394-424.
3. Pourhoseingholi MA, Fazeli Z, Ashtari S, Bavand-Pour FS. Mortality trends of gastrointestinal cancers in Iranian population. *Gastroenterol Hepatol Bed Bench* 2013; 6(Suppl 1): S52-S57.
4. Liu Z, Xu H, You W, Pan K, Li W. *Helicobacter pylori* eradication for primary prevention of gastric cancer: progresses and challenges. *J Natl Cancer Cent* 2024; 4: 299-310.
5. Liou JM, Malfertheiner P, Smith SI, El-Omar EM, Wu MS. 40 years after the discovery of *Helicobacter pylori*: towards elimination of *H. pylori* for gastric cancer prevention. *Lancet* 2024; 403: 2570-2572.
6. Alexander GA, Brawley OW. Association of *Helicobacter pylori* infection with gastric cancer. *Mil Med* 2000; 165: 21-27.
7. Sun K, Jia K, Lv H, Wang SQ, Wu Y, Lei H, et al. EBV-Positive Gastric Cancer: Current Knowledge and Future Perspectives. *Front Oncol* 2020; 10: 583463.
8. Zeng ZM, Luo FF, Zou LX, He RQ, Pan DH, Chen X, et al. Human papillomavirus as a potential risk factor for gastric cancer: a meta-analysis of 1,917 cases. *Oncotargets Ther* 2016; 9: 7105-7114.
9. Mazurek M, Jaros M, Gliwa AM, Sitarz MZ, Dudzińska E, Zinkiewicz K, et al. Epstein-Barr Virus (EBV) and Human Papilloma Virus (HPV) in Gastric Cancers, with Special Reference to Gastric Cancer at a Young Age-A Pilot Study in Poland. *Int J Mol Sci* 2025; 26: 711.
10. de Souza CRT, Almeida MCA, Khayat AS, da Silva EL, Soares PC, Chaves LC, et al. Association between *Helicobacter pylori*, Epstein-Barr virus, human papillomavirus and gastric adenocarcinomas. *World J Gastroenterol* 2018; 24: 4928-4938.
11. Charostad J, Azaran A, Nakhaei M, Astani A, Kaydani GA, Motamedfar A, et al. Upregulation of interleukin-6 in HPV-positive breast cancer patients. *Iran J Immunol* 2021; 18: 315-330.
12. Charostad J, Nakhaei M, Azaran A, Kaydani GA, Astani A, Motamedfar A, et al. MiRNA-218 is frequently downregulated in malignant breast tumors: a footprint of Epstein-Barr virus infection. *Iran J Pathol* 2021; 16: 376-385.
13. Kumar S, Metz DC, Ellenberg S, Kaplan DE, Goldberg DS. Risk factors and incidence of gastric cancer after detection of *Helicobacter pylori* infection: a large cohort study. *Gastroenterology* 2020; 158(3): 527-536.e7.
14. Bae JM. Human papillomavirus infection and gastric

- cancer risk: A meta-epidemiological review. *World J Virol* 2021; 10: 209-216.
15. Saito M, Kono K. Landscape of EBV-positive gastric cancer. *Gastric Cancer* 2021; 24: 983-989.
 16. Shechter O, Sausen DG, Gallo ES, Dahari H, Borenstein R. Epstein-Barr Virus (EBV) Epithelial Associated Malignancies: Exploring Pathologies and Current Treatments. *Int J Mol Sci* 2022; 23: 14389.
 17. Cândido AC, de Lima Filho JL, Martins DB, Mendes CM, Vieira JR, Ferraz AA. Association of human papillomavirus genomic sequences by polymerase chain reaction in gastric carcinomas in Brazil. *Anal Quant Cytopathol Histopathol* 2013; 35: 1-6.
 18. Fakhraei F, Haghshenas MR, Hosseini V, Rafiei A, Naghshvar F, Alizadeh-Navaei R. Detection of human papillomavirus DNA in gastric carcinoma specimens in a high-risk region of Iran. *Biomed Rep* 2016; 5: 371-375.
 19. Jafari-Sales A, Shariat A, Bannazadeh-Baghi H, Baradaran B, Jafari B. Human Papillomavirus (HPV) Prevalence and E6 Protein Expression in Gastric Cancer Tissue Samples Compared with Non-malignant and Control Groups in East Azerbaijan Province, Iran, 2021. *Iran J Med Microbiol* 2023; 17: 58-65.
 20. Cárdenas-Mondragón MG, Torres J, Flores-Luna L, Camorlinga-Ponce M, Carreón-Talavera R, Gomez-Delgado A, et al. Case-control study of Epstein-Barr virus and *Helicobacter pylori* serology in Latin American patients with gastric disease. *Br J Cancer* 2015; 112: 1866-1873.
 21. Singh S, Jha HC. Status of Epstein-Barr Virus Coinfection with *Helicobacter pylori* in Gastric Cancer. *J Oncol* 2017; 2017: 3456264.
 22. Fierti AO, Yakass MB, Okertchiri EA, Adadey SM, Quaye O. The Role of Epstein-Barr Virus in Modulating Key Tumor Suppressor Genes in Associated Malignancies: Epigenetics, Transcriptional, and Post-Translational Modifications. *Biomolecules* 2022; 12: 127.