

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2018 November 21; 24(43): 4835-4958



**EDITORIAL**

- 4835** Promoting genetics in non-alcoholic fatty liver disease: Combined risk score through polymorphisms and clinical variables

*Vespasiani-Gentilucci U, Gallo P, Dell'Unto C, Volpentesta M, Antonelli-Incalzi R, Picardi A*

**REVIEW**

- 4846** Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes

*McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS*

**MINIREVIEWS**

- 4862** Cryotherapy in the management of premalignant and malignant conditions of the esophagus

*Lal P, Thota PN*

- 4870** Acute acalculous cholecystitis in children

*Poddighe D, Sazonov V*

**ORIGINAL ARTICLE****Basic Study**

- 4880** Establishment, functional and genetic characterization of three novel patient-derived rectal cancer cell lines

*Gock M, Mullins CS, Bergner C, Prall F, Ramer R, Göder A, Krämer OH, Lange F, Krause BJ, Klar E, Linnebacher M*

- 4893** Zinc finger E-box-binding homeobox 1 mediates aerobic glycolysis *via* suppression of sirtuin 3 in pancreatic cancer

*Xu WY, Hu QS, Qin Y, Zhang B, Liu WS, Ni QX, Xu J, Yu XJ*

- 4906** Prognostic value of sorting nexin 10 weak expression in stomach adenocarcinoma revealed by weighted gene co-expression network analysis

*Zhang J, Wu Y, Jin HY, Guo S, Dong Z, Zheng ZC, Wang Y, Zhao Y*

**Retrospective Cohort Study**

- 4920** Warm ischemia time and elevated serum uric acid are associated with metabolic syndrome after liver transplantation with donation after cardiac death

*Hu LS, Chai YC, Zheng J, Shi JH, Zhang C, Tian M, Lv Y, Wang B, Jia A*

**Retrospective Study**

- 4928** Association between *Helicobacter pylori*, Epstein-Barr virus, human papillomavirus and gastric adenocarcinomas

*de Souza CR, Almeida MC, Khayat AS, da Silva EL, Soares PC, Chaves LC, Burbano RM*

- 4939** Risk of recurrence of primary sclerosing cholangitis after liver transplantation is associated with *de novo* inflammatory bowel disease

*Bajer L, Slavcev A, Macinga P, Sticova E, Brezina J, Roder M, Janousek R, Trunecka P, Spicak J, Drastich P*

- 4950** Biomarkers and potential pathogenesis of colorectal cancer-related ischemic stroke

*Qin QX, Cheng XM, Lu LZ, Wei YF, Wang DC, Li HH, Li GH, Liang HB, Li SY, Chen L, Liang ZJ*



## Contents

*World Journal of Gastroenterology*  
Volume 24 Number 43 November 21, 2018

### ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Martina Perse, PhD, Associate Research Scientist, Institute of Pathology, Medical Experimental Centre, University of Ljubljana, Faculty of Medicine, Ljubljana 1000, Slovenia

### AIMS AND SCOPE

*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup> each month. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

### INDEXING/ABSTRACTING

*World Journal of Gastroenterology* (*WJG*) is now indexed in Current Contents<sup>®</sup>/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch<sup>®</sup>), Journal Citation Reports<sup>®</sup>, Index Medicus, MEDLINE, PubMed, PubMed Central and Directory of Open Access Journals. The 2018 edition of Journal Citation Reports<sup>®</sup> cites the 2017 impact factor for *WJG* as 3.300 (5-year impact factor: 3.387), ranking *WJG* as 35<sup>th</sup> among 80 journals in gastroenterology and hepatology (quartile in category Q2).

### EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Ying-Na Bian*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Xue-Jiao Wang*  
Proofing Editorial Office Director: *Ze-Mao Gong*

#### NAME OF JOURNAL

*World Journal of Gastroenterology*

#### ISSN

ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

#### LAUNCH DATE

October 1, 1995

#### FREQUENCY

Weekly

#### EDITORS-IN-CHIEF

**Andrzej S Tarnawski, MD, PhD, DSc (Med),**  
**Professor of Medicine, Chief Gastroenterology, VA**  
Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

#### EDITORIAL BOARD MEMBERS

All editorial board members resources online at <http://www.wjgnet.com/1007-9327/editorialboard.htm>

#### EDITORIAL OFFICE

Ze-Mao Gong, Director  
*World Journal of Gastroenterology*  
Baishideng Publishing Group Inc  
7901 Stoneridge Drive, Suite 501,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

#### PUBLISHER

Baishideng Publishing Group Inc  
7901 Stoneridge Drive, Suite 501,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

#### PUBLICATION DATE

November 21, 2018

#### COPYRIGHT

© 2018 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

#### SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

#### INSTRUCTIONS TO AUTHORS

Full instructions are available online at <http://www.wjgnet.com/bpg/gerinfo/204>

#### ONLINE SUBMISSION

<http://www.f6publishing.com>

## Retrospective Study

# Association between *Helicobacter pylori*, Epstein-Barr virus, human papillomavirus and gastric adenocarcinomas

Carolina Rosal Teixeira de Souza, Marcelli Carolini Alves Almeida, André Salim Khayat, Emerson Lucena da Silva, Paulo Cardoso Soares, Luiz Cláudio Chaves, Rommel Mario Rodríguez Burbano

Carolina Rosal Teixeira de Souza, Marcelli Carolini Alves Almeida, André Salim Khayat, Emerson Lucena da Silva, Rommel Mario Rodríguez Burbano, Laboratory of Human Cytogenetics, Institute of Biological Sciences, Federal University of Pará, Belém, Pará 66075-110, Brazil

André Salim Khayat, Rommel Mario Rodríguez Burbano, Oncology Research Center, Federal University of Pará, João de Barros Barreto University Hospital, Belém, Pará 66073-000, Brazil

Paulo Cardoso Soares, Luiz Cláudio Chaves, Rommel Mario Rodríguez Burbano, Ophir Loyola Hospital, Belém, Pará 66060-281, Brazil

ORCID number: Carolina Rosal Teixeira de Souza (0000-0002-5123-9025); Marcelli Carolini Alves Almeida (0000-0002-0670-6608); André Salim Khayat (0000-0002-3451-6369); Emerson Lucena da Silva (0000-0002-5761-8413); Paulo Cardoso Soares (0000-0002-2412-5057); Luiz Cláudio Chaves (0000-0002-0823-245X); Rommel Mario Rodríguez Burbano (0000-0002-4872-234X).

**Author contributions:** de Souza CR and Almeida MC contributed to this work equally, carried out the studies, collected the data, performed the statistical analysis and drafted the manuscript; Khayat AS performed the statistical analysis and participated in its design; da Silva EL performed the statistical analysis and drafted the manuscript; Soares PC, Chaves LC and Burbano RM participated in critical revision and editing of the manuscript; Burbano RM conceptualized and designed the study and helped to draft the manuscript; all authors read and approved the final manuscript.

**Supported by the National Council for scientific and technological development, No. (CNPq) 402283/2013-9.**

**Institutional review board statement:** This study was approved by the Ethics Committee of the João de Barros Barreto University Hospital in Belém, No. 142004 and No. 637.233.

**Informed consent statement:** Patients were not required to give informed consent for the publication because the study and analysis used anonymous data that were obtained after each

patient agreed to the collection of pieces of the tumors by written consent.

**Conflict-of-interest statement:** The authors declare no conflicts of interest.

**Data sharing statement:** The authors agree that if this manuscript is finally accepted for publication, the Copyright License Agreement will become effective immediately.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Corresponding author to:** Carolina Rosal Teixeira de Souza, PhD, Adjunct Professor, Laboratório de Citogenética Humana, Instituto de Ciências Biológicas, Universidade Federal do Pará, Rua Augusto Corrêa, 1 - Guamá, Belém, Pará 66075-110, Brazil. [carolrosalts@gmail.com](mailto:carolrosalts@gmail.com)  
Telephone: +55-91-32018188  
Fax: +55-91-32018188

**Received:** June 8, 2018

**Peer-review started:** June 8, 2018

**First decision:** July 4, 2018

**Revised:** September 11, 2018

**Accepted:** October 5, 2018

**Article in press:** October 5, 2018

**Published online:** November 21, 2018

## Abstract

### AIM

To correlate *Helicobacter pylori* (*H. pylori*), Epstein-

Barr virus (EBV) and human papillomavirus (HPV) with gastric cancer (GC) cases in Pará State, Brazil.

## METHODS

Tissue samples were obtained from 302 gastric adenocarcinomas. A rapid urease test was used to detect the presence of *H. pylori*, and the presence of the *cagA* gene in the HP-positive samples was confirmed by PCR. An RNA *in situ* hybridization test designed to complement Eber1 RNA was used to detect the presence of EBV in the samples, and the L1 region of HPV was detected using nested PCR. Positive HPV samples were genotyped and analyzed for E6 and E7 viral gene expression. Infections were also correlated with the clinical and pathological characteristics of the patients.

## RESULTS

The majority of the 302 samples analyzed were obtained from men (65%) aged 55 years or older (67%) and were classified as the intestinal subtype (55%). All three pathogens were found in the samples analyzed in the present study (*H. pylori*: 87%, EBV: 20%, HPV: 3%). Overall, 78% of the *H. pylori*-positive (*H. pylori*<sup>+</sup>) samples were *cagA*<sup>+</sup> (*H. pylori-cagA*<sup>+</sup>), and there was an association between the cytotoxic product of this gene and EBV. Coinfections of *H. pylori-cagA*<sup>+</sup> and EBV were correlated with the most advanced tumor stages. Although only 20% of the tumors were positive for EBV, infection with this virus was associated with distant metastasis. Only the HPV 16 and 18 strains were found in the samples, although no expression of the E6 and E7 oncoproteins was detected. The fundus of the stomach was the region least affected by the pathogens.

## CONCLUSION

HPV was not involved in gastric tumorigenesis. Prophylactic and therapeutic measures against *H. pylori* and EBV may prevent the development of GC, especially the more aggressive forms.

**Key words:** Gastric cancer; Gastric adenocarcinomas; Microorganisms; *Helicobacter pylori*; *cagA*; Epstein-Barr virus; Human papillomavirus

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We investigated the presence of *Helicobacter pylori* (*H. pylori*), Epstein-Barr virus (EBV) and human papillomavirus in gastric adenocarcinomas and their relationships with the clinicopathological characteristics of the patients. Despite the fact that all three pathogens were found in the samples, we believe that only *H. pylori* and EBV contribute to the transformation of tissue associated with carcinogenesis. A significant association between the *cagA* gene of *H. pylori* and EBV was also observed. Given this finding, the use of prophylactic and therapeutic measures against both *H. pylori* and EBV may help to prevent the development of

gastric cancer, especially the more aggressive forms.

de Souza CR, Almeida MC, Khayat AS, da Silva EL, Soares PC, Chaves LC, Burbano RM. Association between *Helicobacter pylori*, Epstein-Barr virus, and human papillomavirus and gastric adenocarcinomas. *World J Gastroenterol* 2018; 24(43): 4928-4938. Available from: URL: <http://www.wjgnet.com/1007-9327/full/v24/i43/4928.htm> DOI: <http://dx.doi.org/10.3748/wjg.v24.i43.4928>

## INTRODUCTION

Though the worldwide incidence of gastric cancer (GC) has declined rapidly over the past few decades, GC is still the world's fourth leading cause of cancer deaths in both males and females<sup>[1]</sup>. In Brazil, approximately 20 thousand cases of GC are expected in 2018 and 2019; in the North and Northeast regions of the country, it is the second most common type of cancer in men, and it is the fifth most common in women in the North, Southern and Central West regions<sup>[2]</sup>.

The majority of GC cases have a multifactorial origin, that is, they are determined by a combination of genetic and environmental factors. Just over half of all cases of GC are the result of a gradual accumulation of driver mutations caused by environmental factors<sup>[3]</sup>, such as a diet high in salt and carbohydrates, a high intake of food preserved with nitrites, and reduced consumption of fruit and vegetables<sup>[4,5]</sup>. Microbial infections have also been shown to contribute to gastric tumorigenesis<sup>[6-9]</sup>, and gastric physiology and immunology are known to be altered by *Helicobacter pylori* (*H. pylori*)<sup>[10,11]</sup>. While more than half of the world's population is infected with *H. pylori*, however, only approximately 3% of these individuals will develop GC<sup>[12-14]</sup>. One of the factors that may be responsible for this discrepancy is the genetic variability of HP, such as the presence of the *cagA* gene, and interaction with other pathogens<sup>[15-19]</sup>.

The Epstein-Barr virus (EBV) is a second pathogen that may be associated with GC<sup>[7,9]</sup>. This virus provokes a disruption of the genes involved in cell cycle regulation, inflammation, and angiogenesis, as well as the loss of tumor suppressor genes by hypermethylation<sup>[20,21]</sup>.

However, in the case of the third pathogen investigated here, the human papillomavirus (HPV), no systematic association has been established<sup>[6,22-24]</sup>. While the oncogenic properties of HPV have been demonstrated in studies of other portions of the digestive tract<sup>[25-28]</sup>, its possible relationship with GC is still unclear<sup>[23,24,29,30]</sup>.

Given the potential influence of *H. pylori* and EBV on the development of GC<sup>[7,8]</sup> and the high prevalence of HPV in the general population of Pará State, Brazil<sup>[31,32]</sup>, the present study aimed to elucidate the possible relationship of these microorganisms with the clinical-pathological characteristics of patients with GC in this region.

## MATERIALS AND METHODS

### Patients and tissue samples

Tissue samples were obtained from 302 gastric adenocarcinomas collected between 2005 and 2015 in the city of Belém, Pará, Brazil. The patients were between 28 and 92 years old, with a mean age of 62 years. This study was approved by the Ethics Committee of the João de Barros Barreto University Hospital in Belém (No. 142004 and No. 637.233). Written informed consent was obtained from all the patients included in the study prior to the collection of samples. No patient had any previous history of any other type of tumor, and all samples were obtained prior to the administration of chemo- and/or radiotherapy. The tumors were categorized according to the TNM classification system of American Joint Committee on Cancer (AJCC) Cancer Staging<sup>[33]</sup>, the patients' clinical and histopathological analyses, and Lauren's histological classification<sup>[34]</sup>.

### Sample processing

For the molecular analysis, the biological material was frozen in liquid nitrogen directly after collection. DNA was then extracted from the macerated tumor tissue with phenol-chloroform<sup>[35]</sup>, and RNA was obtained using Tri-reagent® (Thermo, CA, United States), following the manufacturer's protocol. cDNA was synthesized by incubating the samples at 37 °C for 1 h in a buffer containing 50 mmol/L Tris pH 8.4, 75 mmol/L KCl, 23 mol/L MgCl<sub>2</sub>, 300 ng of RNA, 0.2 µg of oligo-dT, 10 mmol/L DTT, 0.5 mmol/L dNTP, 10 units of RNase inhibitor, and 50 units of reverse transcriptase. For the anatomopathological analyses, the material was fixed in paraformaldehyde before being embedded in paraffin and stained with hematoxylin and eosin.

### Detection of *H. pylori* and *cagA*

*H. pylori* produces urease to convert urea to ammonia, and this property is exploited by the commercially available rapid urease test (Promedical, Juiz de Fora, Minas Gerais, Brazil) to detect the presence of *H. pylori* in gastric samples. We applied this test to all gastric samples, and when urease was detected, the pH and consequently, the color of the solution, were changed.

The samples were first placed in a tube containing 20 g/L of Christensen's urea agar and incubated at 37 °C for 24 h before being examined for urea hydrolysis. PCR was used to confirm negative results and detect the presence of the *cagA* gene in the *H. pylori*-positive (*H. pylori*<sup>+</sup>) samples. The oligonucleotides used here were described by Covacci *et al.*<sup>[36]</sup>. A sample was considered positive if a clear band was visible in the 20 g/L agarose electrophoresis gel in comparison with a molecular weight marker, and all reactions were performed in duplicate.

### Detection of EBV

EBV is known to infect lymphocytes, but these cells were not included in the present analysis. In this case, EBV was

detected in the gastric samples by using a 30-bp biotinylated probe (5'-AGACACCGTCCTCACCACCCGGGACTTGTA-3') that is complementary to the most abundant viral product in latently infected cells, the EBV-encoded small RNA-1 (Eber1)<sup>[37]</sup>. For this assay, RNA *in situ* hybridization (ISH) was used, and the signal was amplified using a mouse anti-biotin antibody (clone BK, 1:20 dilution; DakoCytomation®, CA, United States) and a biotinylated rabbit anti-immunoglobulin antibody (polyclonal, 1:100 dilution; DakoCytomation®). Streptavidin-biotin peroxidase complex (DakoCytomation®) and diaminobenzidine chromogen (DakoCytomation®) were used to detect this reaction. The slides were counterstained with Harris's hematoxylin, and the cells were examined under light microscopy at 40 × or 20 × magnification by two independent investigators. In this examination, 10 representative microscopic fields containing at least five cells were evaluated. The positive control was an EBV-positive GC sample, and two untreated slides were used as negative controls. Samples were considered positive when 5% or more of the epithelial cells were stained brown/red.

### Detection of HPV

Nested PCR was used to classify the samples as HPV positive or negative. The first PCR used the generic PGMY09/11 primers described by Gravitt *et al.*<sup>[38]</sup>, which amplify the L1 region of HPV, and the second PCR used the GP5+/6+ primers described by Jacobs *et al.*<sup>[39]</sup>. The PCR products were separated on a 2% agarose gel. After identification, the virus was genotyped by sequencing the PCR product with the GP5+ primer using a capillary 3730XL DNA Analyzer (Thermo Fisher Scientific, United States). All the nucleotide sequences obtained in these analyses were compared with the GenBank/NCBI database using the BLAST alignment search tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The E6 and E7 oncoprotein expression of HPV 16 and 18 were investigated by RT-PCR as described by Chang *et al.*<sup>[40]</sup>.

### Statistical analysis

The results of the infections were correlated with the clinical-pathological data of the gastric adenocarcinoma patients. The Shapiro-Wilk test was used to evaluate the distribution of the samples. The association between *H. pylori*, EBV and HPV infections (either separately or combined) and clinical-pathological parameters was evaluated using the chi-square test and logistic regression analysis. A *P* < 0.05 significance level was considered for all analyses, and a 95% confidence interval was also applied. The results are given as the *P* value, odds ratio (OR), and confidence interval (CI). The statistical methods of this study were reviewed by André Salim Khayat from Oncology Research Center, Federal University of Pará.

## RESULTS

The studied population was divided into two age groups:



Table 1 Clinicopathological data of patients with gastric cancer and their relation with the presence or absence of *Helicobacter pylori*, *Helicobacter pylori-cagA*, Epstein-Barr virus and human papillomavirus

	n (%)	HP- 38 (13)	HP+ 264 (87)	P (95%CI)	CagA- 98 (23)	CagA+ 204 (77)	P (95%CI)	EBV- 240 (80)	EBV+ 62 (20)	P (95%CI)	HPV- 294 (97)	HPV+ 8 (3)	P (95%CI)
Gender													
Male	195 (65)	25 (66)	170 (64)	0.866 (0.46-1.92)	60 (61)	135 (66)	0.400 (0.75-2.04)	152 (63)	43 (69)	0.377 (0.72-2.39)	192 (65)	3 (37)	0.105 (0.75-1.36)
Fem	107 (35)	13 (34)	94 (36)		38 (39)	69 (34)		88 (37)	19 (31)		102 (35)	5 (63)	
Age													
≥ 55	201 (67)	24 (63)	177 (67)	0.635 (0.58-2.41)	65 (66)	136 (67)	0.953 (0.61-1.69)	157 (65)	44 (71)	0.409 (0.70-2.38)	201 (68)	0 (0)	0.000 (1.03-1.15)
< 55	101 (33)	14 (37)	87 (33)		33 (34)	68 (33)		83 (35)	18 (29)		93 (32)	8 (100)	
Localization													
Proximal	104 (34)	14 (36)	90 (34)	0.837 (0.46-1.87)	37 (38)	67 (33)	0.400 (0.49-1.33)	81 (34)	23 (37)	0.621 (0.65-2.07)	100 (34)	4 (50)	0.348 (0.47-7.92)
Distal	198 (66)	25 (64)	173 (66)		61 (62)	137 (67)		159 (66)	39 (63)		194 (66)	4 (50)	
Subtype													
Intestinal	166 (55)	14 (37)	152 (58)	0.016 (1.15-4.70)	48 (49)	118 (58)	0.147 (0.88-2.32)	127 (53)	39 (63)	0.159 (0.85-2.70)	164 (56)	2 (25)	0.084 (0.05-1.33)
Diffuse	136 (45)	24 (63)	112 (42)		50 (51)	86 (42)		113 (47)	23 (37)		130 (44)	6 (75)	
TNM													
T													
T1-T2	70 (23)	10 (27)	60 (23)	0.624 (0.38-1.79)	27 (28)	43 (21)	0.212 (0.40-1.22)	56 (23)	14 (23)	0.900 (0.49-1.87)	65 (22)	5 (62)	0.008 (1.37-25.22)
T3-T4	232 (77)	28 (73)	204 (77)		71 (72)	161 (79)		184 (77)	48 (77)		229 (78)	3 (38)	
N													
N0	16 (5)	2 (5)	14 (5)	0.992 (0.22-4.55)	5 (5)	11 (5)	0.916 (0.32-2.79)	13 (5)	3 (5)	0.856 (0.31-4.08)	16 (5)	0 (0)	0.498 (0.92-0.97)
N1-N3	286 (95)	36 (95)	250 (95)		93 (95)	193 (95)		227 (95)	59 (95)		278 (95)	8 (100)	
M													
M0	141 (47)	18 (47)	123 (47)	0.928 (0.52-2.04)	48 (49)	93 (46)	0.580 (0.71-1.86)	121 (50)	20 (32)	0.011 (1.18-3.85)	137 (47)	4 (50)	0.849 (0.21-3.55)
M1	161 (53)	20 (53)	141 (53)		50 (51)	111 (54)		119 (50)	42 (68)		157 (53)	4 (50)	
EBV													
EBV+	62 (20)	7 (18)	55 (21)	0.731 (0.49-2.79)	9 (9)	53 (26)	0.001 (1.63-7.37)						
EBV-	240 (80)	31 (82)	209 (79)		89 (91)	151 (74)							
HPV													
HPV+	8 (3)	0 (0)	8 (3)	0.277 (1.01-1.05)	1 (1)	7 (3)	0.222 (0.42-28.41)	6 (2)	2 (3)	0.751 (0.26-6.60)			
HPV-	294 (97)	38 (100)	256 (97)		97 (99)	197 (97)		234 (98)	60 (97)				

EBV: Epstein-Barr virus; HPV: Human papillomavirus.

Individuals aged 55 years or over (67% of the total) and individuals younger than 55 years (33%). The majority (65%,  $n = 195$ ) of the 302 samples analyzed in the present study were obtained from men, with the remainder (35%,  $n = 107$ ) being obtained from women. The clinicopathological data on the GC patients and their association with the presence or absence of *H. pylori*, EBV and HPV are shown in Table 1.

The GC cases were divided into two groups based on the presence of gastric epithelial stem cells: (1) proximal gastric carcinoma (cardia), and (2) distal carcinoma (noncardia), with specific pathological, clinical, epidemiological and prognostic characteristics<sup>[41]</sup>. Overall, 35% of the tumors were located in the proximal region, and 65% in the distal region (31% in the antrum, 19% in the fundus, and 15% in the body).

Based on Lauren's microscopic classification, the most widely used system for gastric adenocarcinomas, there are two principal types of gastric tumor: (1) the diffuse type, which has a worse prognosis and is characterized by invasive growth and the absence of premalignant lesions, and (2) the intestinal type, the development of which is



**Table 2** Ratio, in absolute value and percentage, between pathogen (*Helicobacter pylori*, Epstein-Barr virus, and human papillomavirus), strain (HPcagA) and tumor location

Infection	Cardia (104)	P (OR, 95%CI)	Fundus (57)	P (OR, 95%CI)	Body (45)	P (OR, 95%CI)	Antrum (95)	P (OR, 95%CI)
HP+ (263)	91 (34.6%)	0.975 (OR = 1.012, 95%CI: 0.494-2.071)	44 (16.7%)	0.012 (OR = 0.395, 95%CI: 0.188-0.831)	40 (15.2%)	0.704 (OR = 1.213, 95%CI: 0.447-3.291)	88 (33.5%)	0.064 (OR = 2.214, 95%CI: 0.938-5.228)
<i>H. pylori</i> -cagA+ (203)	67 (33%)	0.400 (OR = 0.806, 95%CI: 0.488-1.332)	27 (13.3%)	0.000 (OR = 0.361, 95%CI: 0.201-0.648)	35 (17.2%)	0.092 (OR = 1.886, 95%CI: 0.894-3.978)	74 (36.5%)	0.009 (OR = 2.087, 95%CI: 1.191-3.656)
EBV+ (62)	23 (37.1%)	0.621 (OR = 1.158, 95%CI: 0.648-2.069)	1 (1.6%)	0.000 (OR = 0.053, 95%CI: 0.007-0.388)	16 (25.8%)	0.009 (OR = 2.435, 95%CI: 1.227-4.833)	22 (35.5%)	0.444 (OR = 1.258, 95%CI: 0.699-2.266)
HPV+ (8)	4 (50%)	0.348 (OR = 1.940, 95%CI: 0.475-7.920)	0 (0%)	0.162 (OR = 0.803, 95%CI: 0.758-0.850)	2 (25%)	0.436 (OR = 1.894, 95%CI: 0.370-9.686)	2 (25%)	0.690 (OR = 0.720, 95%CI: 0.143-3.637)

EBV: Epstein-Barr virus; HPV: Human papillomavirus; *H. pylori*: *Helicobacter pylori*.

dependent on environmental factors and is associated with premalignant lesions, particularly chronic and atrophic gastritis, metaplasia and dysplasia<sup>[34,42,43]</sup>. According to this classification, 55% (166) of the samples were of the intestinal subtype, and the other 45% (136) of the diffuse subtype. Based on the stages defined by the AJCC (TNM)<sup>[33]</sup>, 77% (232) of the samples had the worst prognosis (T3 and T4), 95% (286) involved lymph nodes (N1, N2, N3), and 53% (161) had distant metastasis (M1).

The *H. pylori* bacterium was detected (*H. pylori*<sup>+</sup>) in 87% ( $n = 264$ ) of the tumor tissue samples, and tumors of the intestinal type were more likely to be infected with *H. pylori* (92%) than those of the diffuse subtype (82%) ( $P = 0.016$ , OR = 2.327, CI: 1.15-4.70). There was no significant relationship between HP infection and the tumor stage, however, nor with the presence of EBV or HPV.

The *cagA* gene (*H. pylori*-cagA<sup>+</sup>) was amplified in 77% (204) of the *H. pylori*<sup>+</sup> samples. The relative frequency of the *H. pylori*-cagA<sup>+</sup> samples did not differ from that of the *H. pylori*-cagA<sup>-</sup> samples by sex, age, subtype or tumor stage. However, while EBV was present in 26% (53) of the *H. pylori*-cagA<sup>+</sup> samples, only 9% (9) of the *H. pylori*-cagA<sup>-</sup> samples were EBV positive ( $P = 0.001$ , OR = 3.471, CI: 1.63-7.37). No association was found between cagA<sup>+</sup> and HPV.

Only 20% of the tumors tested were positive for EBV infection (EBV<sup>+</sup>), and the incidence of the virus did not vary by gender, age, subtype, tumor stage, lymph node invasion or the presence of HPV. There was a significant association between EBV<sup>+</sup> and distant metastasis, however, with 68% (42) of the metastasized tumors being EBV<sup>+</sup> ( $P = 0.011$ , OR = 2.135, CI: 1.18-3.85).

Only 3% (8) of samples were positive for HPV (HPV<sup>+</sup>). All cases of HPV<sup>+</sup> were recorded in young (< 55 years old) patients ( $P = 0.000$ , OR = 16.354, CI: 0.87-0.97), and the majority (63%) were female ( $P > 0.05$ ). The analysis identified two types of high-risk HPV, 16 (50%) and 18 (50%), but with no expression of the E6 and E7 oncoproteins. No significant association was found between HPV<sup>+</sup> and sex, lymph node invasion or distant metastasis. A significant relationship was found, however,

with the tumor stage, with the virus being significantly more frequent in less aggressive tumors and 62% of the HPV<sup>+</sup> samples being classified as T1 and T2 ( $P = 0.008$ , OR = 5.872, CI: 1.37-25.22).

Interestingly, only two GC samples had all three pathogens (*H. pylori*<sup>+</sup>, EBV<sup>+</sup> and HPV<sup>+</sup>), although there was obviously no significant association in this case. The majority (89%; 55) of the EBV<sup>+</sup> cases and all of the HPV<sup>+</sup> cases were coinfecting with *H. pylori*. In addition, 96% (53) of the tumors coinfecting with *H. pylori* and EBV (EBV *H. pylori*-cagA<sup>+</sup>) were *H. pylori*-cagA<sup>+</sup> A strains and thus had a six-fold greater chance of having a more aggressive tumor stage ( $P = 0.010$ , OR = 6.111, CI: 0.04-0.73, power of the test > 0.90), as 83% (44) of EBV *H. pylori*-cagA<sup>+</sup> tumors were T3 and T4.

The regions of the stomach infected and the relationship with the pathogen and strain are shown in Table 2. While fewer tumors affected the body region, the fundus of the stomach was least affected by the infections, including negative associations with the *H. pylori*-cagA<sup>+</sup> strain ( $P = 0.00032$ ) and EBV ( $P = 0.00010$ , with Bonferroni correction in both cases).

## DISCUSSION

Many types of cancer are known to be related to infections by microorganisms<sup>[1,44]</sup>, which may play a role in either the onset of the growth of the cancer cells or their maintenance. While much of the gastrointestinal tract represents a favorable environment for microbial life, this is not the case with the stomach, where any microorganism would need to tolerate extremely acidic conditions, antimicrobial compounds, enzymes, and structural barriers<sup>[13]</sup>. Thus, to colonize the stomach, any pathogen must adapt to an extremely hostile and highly variable environment.

Despite these difficulties, some pathogens are able to establish themselves in the stomach, and their presence has been associated conclusively with the development of GC. *H. pylori*, for example, produces urease to make the gastric environment more basic, facilitating its survival<sup>[14,45]</sup>. However, evidence on the involvement of other agents, such as HPV, in the development of gastric

tumors is still inconclusive<sup>[6,23,24,30]</sup>. As in most studies of GC<sup>[16,46-49]</sup>, the present study recorded a predominance of GC cases in male patients over 55 years of age.

### *H. pylori*

The high prevalence of *H. pylori* in GC tumors has been reported widely, both in Brazil and around the world<sup>[50-54]</sup>. The scenario in Pará is typical<sup>[15,47,55]</sup>, with the prevalence of *H. pylori* in tumor samples being up to nine times higher than that recorded in healthy tissue samples<sup>[16]</sup>. In the present study, *H. pylori* was present in a high percentage of tumor samples, with 87% (263) of the patients being affected by the bacterium.

While the exact mechanism through which *H. pylori* may induce gastric carcinogenesis has not yet been fully elucidated, it is known that the inflammatory process caused by this bacterium, coupled with genetic and epigenetic events in the host, is capable of inducing a cascade of morphological events, including both premalignant and malignant transformations (intestinal or diffuse GC)<sup>[8,42]</sup>. In our study, the presence of the bacterium was more frequent in tumors classified as the intestinal subtype, an association that has also been confirmed in other studies conducted in locations with high GC incidence rates<sup>[16,52,56]</sup>.

The *cagA* gene was recorded in 78% (204) of the 263 *H. pylori*<sup>+</sup> samples investigated in this study, a frequency similar to that recorded in other studies in Pará State. Vinagre *et al.*<sup>[15]</sup> recorded a rate of 73% and found that *cagA* was associated with a more intense inflammatory response and higher levels of DNA damage in epithelial cells. Souza *et al.*<sup>[16]</sup> recorded an even higher rate (88%) and found that *H. pylori-cagA*<sup>+</sup> was associated with both lymph node metastasis and distant metastasis. In Portugal, Nogueira *et al.*<sup>[57]</sup> found that 64% of *H. pylori*<sup>+</sup> samples had *cagA*<sup>+</sup>, which was more closely related to the progression of gastric adenocarcinoma in comparison with samples infected with *H. pylori-cagA*<sup>-</sup> strains. This gene has thus been associated with a higher risk of GC development and worse prognosis in a number of studies. In our study, however, while *H. pylori-cagA*<sup>+</sup> was more frequent in more advanced stages (T3 and T4) and in patients with distant metastasis, there was no significant difference ( $P > 0.05$ ) in comparison with the samples that lacked the *cagA* oncogene.

The *cagA* gene was also significantly associated with the presence of coinfection with EBV (EBV *H. pylori-cagA*<sup>+</sup>) and was present in 85% (53) of the EBV<sup>+</sup> tumors, a frequency close to that recorded by Souza *et al.*<sup>[16]</sup>, who recorded coinfection in 100% of EBV samples. Minoura *et al.*<sup>[58]</sup> concluded that the presence of *H. pylori* supports the reactivation of the virus from its latent state in gastric epithelial cells, while Saiki *et al.*<sup>[59]</sup> proposed that the inflammatory stress generated by this bacterium may attract a greater infiltration of lymphocytes carrying EBV, which increases the possibility of epithelial cells coming into contact with these lymphocytes and thus being infected. EBV may also support *H. pylori*.

Cárdenas-Mondragó *et al.*<sup>[19]</sup> found that EBV act as a cofactor in triggering gastric inflammation together with *H. pylori* in gastric diseases, and Saju *et al.*<sup>[60]</sup> recently discovered that host cell SHP1 phosphatase antagonizes the *H. pylori-cagA* virulence factor. However, coinfection with EBV results in methylation of the SHP1 promoter, keeping *cagA* phosphorylated and thereby allowing the mediation of oncogenic signaling. This evidence indicates that the development and progression of GC in some of the patients analyzed may be influenced by an association between the oncogenic characteristics of EBV and the cytotoxic product of the *H. pylori cagA* gene.

These interactions suggest that the EBV *H. pylori-cagA*<sup>+</sup> coinfection favors tissue malignancy, and the higher rate of EBV *H. pylori-cagA*<sup>+</sup> coinfection observed in patients with more aggressive tumors further reinforces the role of this interaction in the development and/or progression of gastric adenocarcinoma in the patients analyzed.

### EBV

This virus is typically found in approximately 10% of GC cases<sup>[9,16,61,62]</sup>, although in the present study, EBV was present in 20% (62) of the samples, a frequency similar to that found in studies in the United States and Germany (16%-26%)<sup>[9,63,64]</sup>. Lower frequencies have been found in other countries<sup>[65-67]</sup>, however, indicating that the prevalence of EBV may vary widely among geographic regions.

Nogueira *et al.*<sup>[57]</sup>, Lopes *et al.*<sup>[61]</sup>, and Shibata *et al.*<sup>[63]</sup> found that EBV was significantly more prevalent in males and that it was associated (but not significantly) with age, although no association was found with tumor subtype. In our study, even though the virus was more prevalent in men, older (> 55 years) patients and patients with the intestinal type of tumor (Table 1), these relationships were not significant ( $P > 0.05$ ).

It is known that the protein encoded by the EBV *BARF1* gene increases the proliferation of virus-infected cancer cells by increasing the expression of NF- $\kappa$ B and reducing p21, thereby facilitating the development of the process<sup>[68]</sup>. Specifically, monocytes are recruited by vascular endothelial growth factor (VEGF), and tumor associated macrophages (TAM) is induced by granulocyte-macrophage colony-stimulating factor (GM-CSF) in an NF- $\kappa$ B-dependent manner<sup>[69]</sup>.

Expression of the EBV oncogenic proteins LMP1, LMP2A, and LMP2B has also been shown to have a role in the increased capacity of the cancer to spread and migrate. In other types of tumor, the LMP1 protein activates the ERK-MAPK pathway and is capable of inducing motility and increasing the migration rate of epithelial cells in comparison with LMP1-negative cells, as shown by Dawson *et al.*<sup>[70]</sup>. LMP2A also increases motility by targeting EGFR<sup>[71]</sup>, which would account for the lymph node metastasis found in other studies<sup>[72,73]</sup> and the increased probability of distant metastasis found in the

present study.

### HPV

There is no consensus in the literature on the potential association between HPV and GC, and in our study, only 3% of the gastric tumor samples analyzed were infected with HPV. Fakhraei *et al.*<sup>[74]</sup> recorded a similar frequency, 5%, in samples of gastric adenocarcinoma in patients from northern Iran. Much higher frequencies, ranging from 37.5% to 52%, have been found in other studies<sup>[6,23,29,75]</sup>, however. Even so, no HPV was found in the GC samples in some studies<sup>[24,30,76]</sup>. This disparity between studies may be related in part to the variation in the viral detection methods used (ISH, PCR), as well as the methods of collecting and preserving the samples, in addition to the geographic location of the research.

When the viral DNA of HPV 16 and 18 integrates with the host cell genome, the E2 viral gene product is altered. This product has a regulatory role in the transcription of the viral E6 and E7 oncoproteins, which generate genomic instability, resulting in cellular abnormalities and the abolition of cell cycle checkpoints, thereby increasing the risk of accumulated genetic abnormalities. In addition to the lack of an adequate immune response, these abnormalities represent a favorable condition for the development of cancer<sup>[77]</sup>. In our study, two types of high-risk HPV, 16 and 18, were found in the tumors analyzed, although no E6 and E7 expression was observed. As these proteins are usually expressed at the beginning of the infection and reflect the persistence of the virus in the host organism<sup>[77]</sup>, this may be an indication that the presence of the virus in the patients' stomachs was only temporary. These observations, together with previous findings, indicate that the presence of HPV probably had no involvement in the initiation or progression of GC in the patients analyzed in this study.

One important detail from our study is that all HPV<sup>+</sup> tumors were found in younger patients (< 55 years). Studies of low-grade intraepithelial lesions (LSIL)<sup>[78]</sup>, cervical cancer<sup>[79]</sup>, and anal cancer<sup>[80]</sup> have also found a tendency for HPV to occur in younger patients (30–40 years of age), probably indicating a more active sex life, which may increase the chance of exposure to HPV infection.

Furthermore, based on the TNM classification, HPV was found more frequently in less aggressive tumors, with 63% of the HPV<sup>+</sup> samples being classified as T1 or T2, similar to the findings of Anwar *et al.*<sup>[81]</sup> and Zeng *et al.*<sup>[6]</sup>. However, this finding may just be a consequence of the occurrence of less aggressive tumors in younger patients ( $P = 0.000$ , OR = 0.113, CI: 0.062–0.206). There was also no significant association with the presence of lymph node involvement, distal metastasis, or the anatomical location of the tumor, as in previous studies<sup>[23,29,82]</sup>.

### Location of the tumor

Most tumors (65%) were present in noncardia regions,

and the stomach body was the least-affected region. By contrast, previous studies in the same geographic location<sup>[56,83]</sup> found that the stomach fundus was the area least affected by gastric adenocarcinoma. There was no significant difference in infection (*H. pylori*, EBV, HPV) and the presence of antigens (*cagA*) between cardia and noncardia regions. However, other studies have found a negative association between HP infection and GC in the cardia<sup>[84,85]</sup>. Even so, the presence of *H. pylori* strains that carry the pathogenic island *cagPAI* may contribute to an increase in the risk of distal GC<sup>[8,10,14,84]</sup>. In previous studies, the most commonly colonized portion of the stomach was non-cardia region, like the antrum<sup>[16,52]</sup>, which is consistent with our findings (Table 2).

The gastric fundus has one of the lowest rates of infection, and there were negative associations with *H. pylori* ( $P = 0.012$ , OR = 0.395, CI: 0.188–0.831), *H. pylori-cagA* ( $P = 0.000$ , OR = 0.361, CI: 0.201–0.648), and EBV ( $P = 0.000$ , OR = 0.053, CI: 0.007–0.388). The body was also associated with relatively few infections, and the lower rates of infection may be related to the fact that these two stomach regions (fundus and body) have a more acidic pH in comparison with the rest of the stomach<sup>[86]</sup>, which protects them against the establishment of infectious microorganisms. Interestingly, EBV was more frequent in tumors of the cardia, in contrast with a study by The Cancer Genome Atlas (TCGA)<sup>[8]</sup>, which showed that most EBV-positive tumors were present in the gastric fundus or body. HPV was not associated with any specific gastric region.

Overall, then, our findings lead us to believe that only *H. pylori* and EBV contribute actively to the transformation of tissue associated with carcinogenesis. In this case, the systematic application of prophylactic and therapeutic measures against *H. pylori* and EBV may help prevent the development of GC and more aggressive tumors.

## ARTICLE HIGHLIGHTS

### Research background

Gastric cancer (GC) is the world's fourth leading cause of cancer deaths, and microbial infections have been shown to contribute to gastric tumorigenesis.

### Research motivation

Gastric physiology and immunology are known to be altered by *Helicobacter pylori* (*H. pylori*) and Epstein-Barr virus (EBV), although there is still doubt about the association of GC with some pathogens, such as human papillomavirus (HPV).

### Research objectives

The present study aimed to elucidate the possible relationship of these microorganisms with the clinical-pathological characteristics of patients with GC in the North region of Brazil.

### Research methods

A total of 302 gastric adenocarcinomas were collected between 2005 and 2015. Patient samples were categorized according to the TNM classification system and by histology. Molecular techniques were used for pathogen investigations, as they are more sensitive.



## Research results

All three pathogens were found in the samples; however, active HPV infection was not identified. Coinfections of *H. pylori-cagA*<sup>+</sup> and EBV were correlated with tumors at the most advanced stages.

## Research conclusions

HPV was not involved in gastric tumorigenesis. On the other hand, *H. pylori* and EBV seem to be directly related to the development and severity of tumors, especially when coinfections exist.

## Research perspectives

Prophylactic and therapeutic measures against *H. pylori* and EBV may prevent the development of GC, especially the more aggressive forms.

## ACKNOWLEDGMENTS

We thank PROPESP/UFPA for assistance with the publication.

## REFERENCES

- 1 **World Health Organization.** Cancer. 1 Feb 2018. Available from: URL: <http://www.who.int/mediacentre/factsheets/fs297/en/>
- 2 Instituto Nacional de Cancer José Alencar Gomes da Silva/Ministério da Saúde. Estimativa 2018: incidencia de cancer no Brasil. 02 Feb 2018. Available from: URL: <http://www.inca.gov.br/estimativa/2018/estimativa-2018.pdf>
- 3 **Tomasetti C**, Li L, Vogelstein B. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. *Science* 2017; **355**: 1330-1334 [PMID: 28336671 DOI: 10.1126/science.aaf9011]
- 4 **Kobayashi J.** Effect of diet and gut environment on the gastrointestinal formation of N-nitroso compounds: A review. *Nitric Oxide* 2018; **73**: 66-73 [PMID: 28587887 DOI: 10.1016/j.niox.2017.06.001]
- 5 **Resende AL**, Mattos IE, Koifman S. Dieta e câncer gástrico: aspectos históricos associados ao padrão de consumo alimentar no estado do Pará. *Revista de Nutrição* 2006; **19**: 511-519 [DOI: 10.1590/S1415-52732006000400010]
- 6 **Zeng ZM**, Luo FF, Zou LX, He RQ, Pan DH, Chen X, Xie TT, Li YQ, Peng ZG, Chen G. Human papillomavirus as a potential risk factor for gastric cancer: a meta-analysis of 1,917 cases. *Oncotargets Ther* 2016; **9**: 7105-7114 [PMID: 27895502 DOI: 10.2147/OTT.S115053]
- 7 **Cancer Genome Atlas Research Network.** Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]
- 8 **Peek RM Jr**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37 [PMID: 11902583 DOI: 10.1038/nrc703]
- 9 **Takada K.** Epstein-Barr virus and gastric carcinoma. *Mol Pathol* 2000; **53**: 255-261 [PMID: 11091849 DOI: 10.1136/mp.53.5.255]
- 10 **Wroblewski LE**, Peek RM Jr, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clin Microbiol Rev* 2010; **23**: 713-739 [PMID: 20930071 DOI: 10.1128/CMR.00011-10]
- 11 **Zhang C**, Powell SE, Betel D, Shah MA. The Gastric Microbiome and Its Influence on Gastric Carcinogenesis: Current Knowledge and Ongoing Research. *Hematol Oncol Clin North Am* 2017; **31**: 389-408 [PMID: 28501083 DOI: 10.1016/j.hoc.2017.01.002]
- 12 **Das JC**, Paul N. Epidemiology and pathophysiology of Helicobacter pylori infection in children. *Indian J Pediatr* 2007; **74**: 287-290 [PMID: 17401270 DOI: 10.1007/s12098-007-0046-6]
- 13 **Shah MA.** Gastric cancer: The gastric microbiota - bacterial diversity and implications. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 692-693 [PMID: 29042691 DOI: 10.1038/nrgastro.2017.140]
- 14 **Peek RM Jr**, Crabtree JE. Helicobacter infection and gastric neoplasia. *J Pathol* 2006; **208**: 233-248 [PMID: 16362989 DOI: 10.1002/path.1868]
- 15 **Vinagre RM**, Corvelo TC, Arnaud VC, Leite AC, Barile KA, Martins LC. Determination of strains of Helicobacter pylori and of polymorphism in the interleukin-8 gene in patients with stomach cancer. *Arq Gastroenterol* 2011; **48**: 46-51 [PMID: 21537542 DOI: 10.1590/S0004-28032011000100010]
- 16 **de Souza CR**, de Oliveira KS, Ferraz JJ, Leal MF, Calcagno DQ, Seabra AD, Khayat AS, Montenegro RC, Alves AP, Assumpção PP, Smith MC, Burbano RR. Occurrence of Helicobacter pylori and Epstein-Barr virus infection in endoscopic and gastric cancer patients from Northern Brazil. *BMC Gastroenterol* 2014; **14**: 179 [PMID: 25318991 DOI: 10.1186/1471-230X-14-179]
- 17 **Brito CA**, Silva LM, Jucá N, Leal NC, de Souza W, Queiroz D, Cordeiro F, Silva NL. Prevalence of cagA and vacA genes in isolates from patients with Helicobacter pylori-associated gastroduodenal diseases in Recife, Pernambuco, Brazil. *Mem Inst Oswaldo Cruz* 2003; **98**: 817-821 [PMID: 14595461 DOI: 10.1590/S0074-02762003000600018]
- 18 **Nogueira C**, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plaisier AP, Quint WG, Ruiz B, Correa P, van Doorn LJ. Helicobacter pylori genotypes may determine gastric histopathology. *Am J Pathol* 2001; **158**: 647-654 [PMID: 11159201 DOI: 10.1016/S0002-9440(10)64006-0]
- 19 **Cárdenas-Mondragón MG**, Torres J, Flores-Luna L, Camorlinga-Ponce M, Carreón-Talavera R, Gomez-Delgado A, Kasamatsu E, Fuentes-Pananá EM. 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 [PMID: 25996206 DOI: 10.1038/bjc.2015.175]
- 20 **Ryan JL**, Jones RJ, Kenney SC, Rivenbark AG, Tang W, Knight ER, Coleman WB, Gulley ML. Epstein-Barr virus-specific methylation of human genes in gastric cancer cells. *Infect Agent Cancer* 2010; **5**: 27 [PMID: 21194482 DOI: 10.1186/1750-9378-5-27]
- 21 **Zhao J**, Liang Q, Cheung KF, Kang W, Lung RW, Tong JH, To KF, Sung JJ, Yu J. Genome-wide identification of Epstein-Barr virus-driven promoter methylation profiles of human genes in gastric cancer cells. *Cancer* 2013; **119**: 304-312 [PMID: 22833454 DOI: 10.1002/cncr.27724]
- 22 **Li TH**, Qin Y, Sham PC, Lau KS, Chu KM, Leung WK. Alterations in Gastric Microbiota After H. Pylori Eradication and in Different Histological Stages of Gastric Carcinogenesis. *Sci Rep* 2017; **7**: 44935 [PMID: 28322295 DOI: 10.1038/srep44935]
- 23 **Ma TY**, Liu WK, Chu YL, Jiang XY, An Y, Zhang MP, Zheng JW. Detection of human papillomavirus type 16 DNA in formalin-fixed, paraffin-embedded tissue specimens of gastric carcinoma. *Eur J Gastroenterol Hepatol* 2007; **19**: 1090-1096 [PMID: 17998834 DOI: 10.1097/MEG.0b013e3282eeb4dc]
- 24 **Koshiol J**, Wei WQ, Kreimer AR, Ren JS, Gravitt P, Chen W, Kim E, Abnet CC, Zhang Y, Kamangar F, Lin DM, Wang GQ, Roth MJ, Dong ZW, Taylor PR, Qiao YL, Dawsey SM. The gastric cardia is not a target for human papillomavirus-induced carcinogenesis. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1137-1139 [PMID: 20332262 DOI: 10.1158/1055-9965.EPI-10-0089]
- 25 **Śnietura M**, Jaworska M, Piękowski W, Goraj-Zajac A, Woźniak G, Lange D. High-risk HPV DNA status and p16 (INK4a) expression as prognostic markers in patients with squamous cell cancer of oral cavity and oropharynx. *Pol J Pathol* 2010; **61**: 133-139 [PMID: 21225495]
- 26 **Steenbergen RD**, de Wilde J, Wilting SM, Brink AA, Snijders PJ, Meijer CJ. HPV-mediated transformation of the anogenital tract. *J Clin Virol* 2005; **32** Suppl 1: S25-S33 [PMID: 15753009 DOI: 10.1016/j.jcv.2004.11.019]
- 27 **Herrero R**, Castellsagué X, Pawlita M, Lissowska J, Kee F, Balaran P, Rajkumar T, Sridhar H, Rose B, Pintos J, Fernández L, Idris A, Sánchez MJ, Nieto A, Talamini R, Tavani A, Bosch FX, Reidel U, Snijders PJ, Meijer CJ, Viscidi R, Muñoz N, Franceschi S; IARC Multicenter Oral Cancer Study Group. Human



- papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst* 2003; **95**: 1772-1783 [PMID: 14652239 DOI: 10.1093/jnci/djg107]
- 28 **Ribeiro MG**, Marcolino LD, Ramos BR, Miranda EA, Trento CL, Jain S, Gurgel RQ, Silva MG, Dolabella SS. High prevalence of human papillomavirus (HPV) in oral mucosal lesions of patients at the Ambulatory of Oral Diagnosis of the Federal University of Sergipe, Northeastern Brazil. *J Appl Oral Sci* 2017; **25**: 69-74 [PMID: 28198978 DOI: 10.1590/1678-77572016-0313]
  - 29 **Xu WG**, Zhang LJ, Lu ZM, Li JY, Ke Y, Xu GW. Detection of human papillomavirus type 16 E6 mRNA in carcinomas of upper digestive tract. *Zhonghua Yi Xue Za Zhi* 2003; **83**: 1910-1914 [PMID: 14642078]
  - 30 **Yuan XY**, Wang MY, Wang XY, Chang AY, Li J. Non-detection of Epstein-Barr virus and Human Papillomavirus in a region of high gastric cancer risk indicates a lack of a role for these viruses in gastric carcinomas. *Genet Mol Biol* 2013; **36**: 183-184 [PMID: 23885199 DOI: 10.1590/S1415-47572013005000018]
  - 31 **Pinto DS**, Fuzii HT, Quaresma JAS. Prevalência de infecção genital pelo HPV em populações urbana e rural da Amazônia Oriental Brasileira. *Cad. Saúde Pública* 2011; **27**: 769-778 [DOI: 10.1590/S0102-311X2011000400016]
  - 32 **de Almeida LM**, Martins LFL, Pontes VB, Corrêa FM, Montenegro RC, Pinto LC, Soares BM, Vidal JPCB, Félix SP, Bertoni N, Szklo M, Moreira MAM. Human Papillomavirus Genotype Distribution among Cervical Cancer Patients prior to Brazilian National HPV Immunization Program. *J Environ Public Health* 2017; **2017**: 1645074 [PMID: 28512474 DOI: 10.1155/2017/1645074]
  - 33 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]
  - 34 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675 DOI: 10.1111/apm.1965.64.1.31]
  - 35 **Sambrook J**, Green MR. Molecular Cloning: A Laboratory Manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1987
  - 36 **Covacci A**, Rappuoli R. PCR amplification of gene sequences from *Helicobacter pylori* strains. In: Lee A, Megraud F (Eds). *Helicobacter pylori* techniques for clinical diagnosis and basic research. Philadelphia: WB Saunders, 1996: 95-109
  - 37 **Bacchi CE**, Bacchi MM, Rabenhorst SH, Soares FA, Fonseca LE Jr, Barbosa HS, Weiss LM, Gown AM. AIDS-related lymphoma in Brazil. Histopathology, immunophenotype, and association with Epstein-Barr virus. *Am J Clin Pathol* 1996; **105**: 230-237 [PMID: 8607450 DOI: 10.1093/ajcp/105.2.230]
  - 38 **Gravitt PE**, Peyton CL, Alessi TQ, Wheeler CM, Coutlée F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ. Improved amplification of genital human papillomaviruses. *J Clin Microbiol* 2000; **38**: 357-361 [PMID: 10618116]
  - 39 **Jacobs MV**, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol* 1997; **35**: 791-795 [PMID: 9041439]
  - 40 **Chang JT**, Kuo TF, Chen YJ, Chiu CC, Lu YC, Li HF, Shen CR, Cheng AJ. Highly potent and specific siRNAs against E6 or E7 genes of HPV16- or HPV18-infected cervical cancers. *Cancer Gene Ther* 2010; **17**: 827-836 [PMID: 20885450 DOI: 10.1038/cgt.2010.38]
  - 41 **Huang Q**, Fang C, Shi J, Sun Q, Wu H, Gold JS, Weber HC, Guan W, Zhang Y, Yu C, Zou X, Mashimo H. Differences in Clinicopathology of Early Gastric Carcinoma between Proximal and Distal Location in 438 Chinese Patients. *Sci Rep* 2015; **5**: 13439 [PMID: 26310451 DOI: 10.1038/srep13439]
  - 42 **Nardone G**, Rocco A, Malfertheiner P. Review article: helicobacter pylori and molecular events in precancerous gastric lesions. *Aliment Pharmacol Ther* 2004; **20**: 261-270 [PMID: 15274662 DOI: 10.1111/j.1365-2036.2004.02075.x]
  - 43 **Watanabe M**, Kato J, Inoue I, Yoshimura N, Yoshida T, Mukoubayashi C, Deguchi H, Enomoto S, Ueda K, Maekita T, Iguchi M, Tamai H, Utsunomiya H, Yamamichi N, Fujishiro M, Iwane M, Tekeshita T, Mohara O, Ushijima T, Ichinose M. Development of gastric cancer in nonatrophic stomach with highly active inflammation identified by serum levels of pepsinogen and *Helicobacter pylori* antibody together with endoscopic rugal hyperplastic gastritis. *Int J Cancer* 2012; **131**: 2632-2642 [PMID: 22383377 DOI: 10.1002/ijc.27514]
  - 44 **Jacqueline C**, Tasiemski A, Sorci G, Ujvari B, Maachi F, Missé D, Renaud F, Ewald P, Thomas F, Roche B. Infections and cancer: the “fifty shades of immunity” hypothesis. *BMC Cancer* 2017; **17**: 257 [PMID: 28403812 DOI: 10.1186/s12885-017-3234-4]
  - 45 **Suerbaum S**, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002; **347**: 1175-1186 [PMID: 12374879 DOI: 10.1056/NEJMra020542]
  - 46 **Anauate AC**, Leal MF, Wisniewski F, Santos LC, Giguek CO, Chen ES, Geraldís JC, Calcagno DQ, Assumpção PP, Demachki S, Arasaki CH, Lourenço LG, Artigiani R, Burbano RR, Smith MAC. Identification of suitable reference genes for miRNA expression normalization in gastric cancer. *Gene* 2017; **621**: 59-68 [PMID: 28411081 DOI: 10.1016/j.gene.2017.04.016]
  - 47 **Araújo TM**, Seabra AD, Lima EM, Assumpção PP, Montenegro RC, Demachki S, Burbano RM, Khayat AS. Recurrent amplification of RTEL1 and ABCA13 and its synergistic effect associated with clinicopathological data of gastric adenocarcinoma. *Mol Cytogenet* 2016; **9**: 52 [PMID: 27366209 DOI: 10.1186/s13039-016-0260-x]
  - 48 **Mabula JB**, McHembe MD, Koy M, Chalya PL, Massaga F, Rambau PF, Masalu N, Jaka H. Gastric cancer at a university teaching hospital in northwestern Tanzania: a retrospective review of 232 cases. *World J Surg Oncol* 2012; **10**: 257 [PMID: 23181624 DOI: 10.1186/1477-7819-10-257]
  - 49 **de Souza CR**, Leal MF, Calcagno DQ, Costa Sozinho EK, Borges Bdo N, Montenegro RC, Dos Santos AK, Dos Santos SE, Ribeiro HF, Assumpção PP, de Arruda Cardoso Smith M, Burbano RR. MYC deregulation in gastric cancer and its clinicopathological implications. *PLoS One* 2013; **8**: e64420 [PMID: 23717612 DOI: 10.1371/journal.pone.0064420]
  - 50 **Batista SA**, Rocha GA, Rocha AM, Saraiva IE, Cabral MM, Oliveira RC, Queiroz DM. Higher number of *Helicobacter pylori* CagA EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. *BMC Microbiol* 2011; **11**: 61 [PMID: 21435255 DOI: 10.1186/1471-2180-11-61]
  - 51 **Roque JRDS**, Machado RS, Rodrigues D, Rech P, Kawakami E. Prevalência de infecção por *Helicobacter pylori* em uma comunidade indígena em São Paulo e fatores associados: estudo transversal. Prevalence of *Helicobacter pylori* infection in an indigenous community in São Paulo and associated factors: cross-sectional study. *Sao Paulo Med J* 2017; **135**: 140-145 [PMID: 28538867 DOI: 10.1590/1516-3180.2016.0114091216]
  - 52 **Elzouki AN**, Buhjab SI, Alkialani A, Habel S, Sasco AJ. Gastric cancer and *Helicobacter pylori* infection in the eastern Libya: a descriptive epidemiological study. *Arab J Gastroenterol* 2012; **13**: 85-88 [PMID: 22980598 DOI: 10.1016/j.ajg.2012.06.002]
  - 53 **Kim J**, Cho YA, Choi IJ, Lee YS, Kim SY, Shin A, Cho SJ, Kook MC, Nam JH, Ryu KW, Lee JH, Kim YW. Effects of interleukin-10 polymorphisms, *Helicobacter pylori* infection, and smoking on the risk of noncardia gastric cancer. *PLoS One* 2012; **7**: e29643 [PMID: 22235320 DOI: 10.1371/journal.pone.0029643]
  - 54 **Abdi E**, Latifi-Navid S, Zahri S, Yazdanbod A, Safaralizadeh R. *Helicobacter pylori* genotypes determine risk of non-cardia gastric cancer and intestinal- or diffuse-type GC in Ardabil: A very high-risk area in Northwestern Iran. *Microb Pathog* 2017; **107**: 287-292 [PMID: 28390977 DOI: 10.1016/j.micpath.2017.04.007]
  - 55 **Calcagno DQ**, Freitas VM, Leal MF, de Souza CR, Demachki S, Montenegro R, Assumpção PP, Khayat AS, Smith Mde A, dos Santos AK, Burbano RR. MYC, FBXW7 and TP53 copy number variation and expression in gastric cancer. *BMC Gastroenterol*

- 2013; **13**: 141 [PMID: 24053468 DOI: 10.1186/1471-230X-13-141]
- 56 **Vinagre RM**, Campos BP, Sousa RM. Case study of stomach adenocarcinoma conducted at a cancer referral hospital in northern Brazil. *Arq Gastroenterol* 2012; **49**: 125-129 [PMID: 22766999 DOI: 10.1590/S0004-28032012000200006]
- 57 **Nogueira C**, Mota M, Gradiz R, Cipriano MA, Caramelo F, Cruz H, Alarcão A, E Sousa FC, Oliveira F, Martinho F, Pereira JM, Figueiredo P, Leitão M. Prevalence and characteristics of Epstein-Barr virus-associated gastric carcinomas in Portugal. *Infect Agent Cancer* 2017; **12**: 41 [PMID: 28814970 DOI: 10.1186/s13027-017-0151-8]
- 58 **Minoura-Etoh J**, Gotoh K, Sato R, Ogata M, Kaku N, Fujioka T, Nishizono A. Helicobacter pylori-associated oxidant monochloramine induces reactivation of Epstein-Barr virus (EBV) in gastric epithelial cells latently infected with EBV. *J Med Microbiol* 2006; **55**: 905-911 [PMID: 16772418 DOI: 10.1099/jmm.0.46580-0]
- 59 **Saiki Y**, Ohtani H, Naito Y, Miyazawa M, Nagura H. Immunophenotypic characterization of Epstein-Barr virus-associated gastric carcinoma: massive infiltration by proliferating CD8+ T-lymphocytes. *Lab Invest* 1996; **75**: 67-76 [PMID: 8683941]
- 60 **Saju P**, Murata-Kamiya N, Hayashi T, Senda Y, Nagase L, Noda S, Matsusaka K, Funata S, Kunita A, Urabe M, Seto Y, Fukayama M, Kaneda A, Hatakeyama M. Host SHP1 phosphatase antagonizes Helicobacter pylori CagA and can be downregulated by Epstein-Barr virus. *Nat Microbiol* 2016; **1**: 16026 [PMID: 27572445 DOI: 10.1038/nmicrobiol.2016.26]
- 61 **Lopes LF**, Bacchi MM, Elgui-de-Oliveira D, Zanati SG, Alvarenga M, Bacchi CE. Epstein-Barr virus infection and gastric carcinoma in São Paulo State, Brazil. *Braz J Med Biol Res* 2004; **37**: 1707-1712 [PMID: 15517087 DOI: 10.1590/S0100-879X2004001100016]
- 62 **Liang Q**, Yao X, Tang S, Zhang J, Yau TO, Li X, Tang CM, Kang W, Lung RW, Li JW, Chan TF, Xing R, Lu Y, Lo KW, Wong N, To KF, Yu C, Chan FK, Sung JJ, Yu J. Integrative identification of Epstein-Barr virus-associated mutations and epigenetic alterations in gastric cancer. *Gastroenterology* 2014; **147**: 1350-1362.e4 [PMID: 25173755 DOI: 10.1053/j.gastro.2014.08.036]
- 63 **Shibata D**, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. *Am J Pathol* 1992; **140**: 769-774 [PMID: 1314023]
- 64 **Salyakina D**, Tsinoremas NF. Viral expression associated with gastrointestinal adenocarcinomas in TCGA high-throughout sequencing data. *Hum Genomics* 2013; **7**: 23 [PMID: 24279398 DOI: 10.1186/1479-7364-7-23]
- 65 **Koriyama C**, Akiba S, Iriya K, Yamaguti T, Hamada GS, Itoh T, Eizuru Y, Aikou T, Watanabe S, Tsugane S, Tokunaga M. Epstein-Barr virus-associated gastric carcinoma in Japanese Brazilians and non-Japanese Brazilians in São Paulo. *Jpn J Cancer Res* 2001; **92**: 911-917 [PMID: 11572757 DOI: 10.1111/j.1349-7006.2001.tb01180.x]
- 66 **Tokunaga M**, Land CE, Uemura Y, Tokudome T, Tanaka S, Sato E. Epstein-Barr virus in gastric carcinoma. *Am J Pathol* 1993; **143**: 1250-1254 [PMID: 8238241]
- 67 **Choi E**, Byeon SJ, Kim SH, Lee HJ, Kwon HJ, Ahn H, Kim DH, Chang MS. Implication of Leptin-Signaling Proteins and Epstein-Barr Virus in Gastric Carcinomas. *PLoS One* 2015; **10**: e0130839 [PMID: 26147886 DOI: 10.1371/journal.pone.0130839]
- 68 **Chang MS**, Kim DH, Roh JK, Middeldorp JM, Kim YS, Kim S, Han S, Kim CW, Lee BL, Kim WH, Woo JH. Epstein-Barr virus-encoded BARF1 promotes proliferation of gastric carcinoma cells through regulation of NF- $\kappa$ B. *J Virol* 2013; **87**: 10515-10523 [PMID: 23824821 DOI: 10.1128/JVI.00955-13]
- 69 **Huang D**, Song SJ, Wu ZZ, Wu W, Cui XY, Chen JN, Zeng MS, Su SC. Epstein-Barr Virus-Induced VEGF and GM-CSF Drive Nasopharyngeal Carcinoma Metastasis via Recruitment and Activation of Macrophages. *Cancer Res* 2017; **77**: 3591-3604 [PMID: 28484077 DOI: 10.1158/0008-5472.CAN-16-2706]
- 70 **Dawson CW**, Laverick L, Morris MA, Tramoutanis G, Young LS. Epstein-Barr virus-encoded LMP1 regulates epithelial cell motility and invasion via the ERK-MAPK pathway. *J Virol* 2008; **82**: 3654-3664 [PMID: 18199641 DOI: 10.1128/JVI.01888-07]
- 71 **Liang J**, Zheng S, Xiao X, Wei J, Zhang Z, Ernberg I, Matskova L, Huang G, Zhou X. Epstein-Barr virus-encoded LMP2A stimulates migration of nasopharyngeal carcinoma cells via the EGFR/Ca<sup>2+</sup>/calpain/ITGB4 axis. *Biol Open* 2017; **6**: 914-922 [PMID: 28512118 DOI: 10.1242/bio.024646]
- 72 **Begnami MD**, Montagnini AL, Vettore AL, Nonogaki S, Brait M, Simoes-Sato AY, Seixas AQ, Soares FA. Differential expression of apoptosis related proteins and nitric oxide synthases in Epstein Barr associated gastric carcinomas. *World J Gastroenterol* 2006; **12**: 4959-4965 [PMID: 16937490 DOI: 10.3748/wjg.v12.i31.4959]
- 73 **Truong CD**, Feng W, Li W, Khoury T, Li Q, Alrawi S, Yu Y, Xie K, Yao J, Tan D. Characteristics of Epstein-Barr virus-associated gastric cancer: a study of 235 cases at a comprehensive cancer center in U.S.A. *J Exp Clin Cancer Res* 2009; **28**: 14 [PMID: 19192297 DOI: 10.1186/1756-9966-28-14]
- 74 **Fakhraei F**, Haghsheenas MR, Hosseini V, Rafiei A, Naghshevar 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 [PMID: 27588180 DOI: 10.3892/br.2016.728]
- 75 **Erol D**, Bulut Y, Yüce H, Ozercan IH. [Investigation of the presence of human papillomavirus DNA in various gastrointestinal carcinoma samples]. *Mikrobiyol Bul* 2009; **43**: 259-268 [PMID: 19621611]
- 76 **Snietura M**, Waniczek D, Piglowski W, Kopec A, Nowakowska-Zajdel E, Lorenc Z, Muc-Wierzgon M. Potential role of human papilloma virus in the pathogenesis of gastric cancer. *World J Gastroenterol* 2014; **20**: 6632-6637 [PMID: 24914388 DOI: 10.3748/wjg.v20.i21.6632]
- 77 **Graham SV**. Human papillomavirus: gene expression, regulation and prospects for novel diagnostic methods and antiviral therapies. *Future Microbiol* 2010; **5**: 1493-1506 [PMID: 21073310 DOI: 10.2217/fmb.10.107]
- 78 **Taghizadeh E**, Taheri F, Abdolkarimi H, Ghorbani Renani P, Gheibi Hayat SM. Distribution of Human Papillomavirus Genotypes among Women in Mashhad, Iran. *Intervirol* 2017; **60**: 38-42 [PMID: 28723690 DOI: 10.1159/000477848]
- 79 **Alberts CJ**, Michel A, Bruisten S, Snijder MB, Prins M, Waterboer T, Schim van der Loeff MF. High-risk human papillomavirus seroprevalence in men and women of six different ethnicities in Amsterdam, the Netherlands: The HELIUS study. *Papillomavirus Res* 2017; **3**: 57-65 [PMID: 28720457 DOI: 10.1016/j.pvr.2017.01.003]
- 80 **Poynten IM**, Tabrizi SN, Jin F, Templeton DJ, Machalek DA, Cornall A, Phillips S, Fairley CK, Garland SM, Law C, Carr A, Hillman RJ, Grulich AE; SPANC Study Team. Vaccine-preventable anal human papillomavirus in Australian gay and bisexual men. *Papillomavirus Res* 2017; **3**: 80-84 [PMID: 28720461 DOI: 10.1016/j.pvr.2017.02.003]
- 81 **Anwar K**, Nakakuki K, Imai H, Inuzuka M. Infection of human papillomavirus (hvp) and epstein-barr-virus (ebv) and p53 overexpression in human gastric-carcinoma. *Int J Oncol* 1995; **7**: 391-397 [PMID: 21552853 DOI: 10.3892/ijo.7.2.391]
- 82 **Ding GC**, Ren JL, Chang FB, Li JL, Yuan L, Song X, Zhou SL, Guo T, Fan ZM, Zeng Y, Wang LD. Human papillomavirus DNA and P16(INK4A) expression in concurrent esophageal and gastric cardia cancers. *World J Gastroenterol* 2010; **16**: 5901-5906 [PMID: 21155014 DOI: 10.3748/wjg.v16.i46.5901]
- 83 **Pereira LP**, Waisberg J, André EA, Zanoto A, Mendes Júnior JP, Soares HP. Detection of Helicobacter pylori in gastric cancer. *Arq Gastroenterol* 2001; **38**: 240-246 [PMID: 12068534 DOI: 10.1590/S0004-28032001000400006]
- 84 **Kamangar F**, Dawsey SM, Blaser MJ, Perez-Perez GI, Pietinen P, Newschaffer CJ, Abnet CC, Albanes D, Virtamo J, Taylor PR. Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with Helicobacter pylori seropositivity. *J Natl Cancer Inst* 2006; **98**: 1445-1452 [PMID: 17047193 DOI: 10.1093/jnci/djj393]

- 85 **Knekt P**, Teppo L, Aromaa A, Rissanen H, Kosunen TU. Helicobacter pylori IgA and IgG antibodies, serum pepsinogen I and the risk of gastric cancer: changes in the risk with extended follow-up period. *Int J Cancer* 2006; **119**: 702-705 [PMID: 16496404 DOI: 10.1002/ijc.21884]
- 86 **Braga LLBC**, Rocha GA, Rocha AMC, Queiroz DMM, de Magalhães DM. Fundamentos da Fisiopatologia da Úlcera Péptica e do Câncer Gástrico. In: Orlá RB, Brito GAC. Sistema Digestório: Integração Básico-Clinica. São Paulo: Blucher, 2016: 731-750 [DOI: 10.5151/9788580391893-27]

**P- Reviewer:** Bang CS, Shi Q    **S- Editor:** Gong ZM    **L- Editor:** A  
**E- Editor:** Bian YN





Published by **Baishideng Publishing Group Inc**  
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>



ISSN 1007-9327

