Gastric Cancer and Associated Pathogens: Is There Any Association in Moroccan Region?

Samia Alaoui Boukhris

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Mounia El khadir

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Safae Karim

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Tiatou Souho

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Dafr-Allah Benajah

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Sidi Adil Ibrahimi

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Laila Chbani

Hassan II University Hospital in Fez: Centre Hospitalier Universitaire Hassan II - Fes

Mohamed El Abkari

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Bahia Bennani (✉ bahia.bennani@usmba.ac.ma)

Sidi Mohamed Ben Abdellah University Faculty of Medicine and Pharmacy: Universite Sidi Mohamed Ben Abdellah Faculte de Medecine et de Pharmacie

Research Article

Keywords: H. pylori, EBV, HPV, gastric cancer, co-infection

Posted Date: December 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1169749/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Purpose: *Helicobacter pylori*, *Epstein-Barr virus* and human papillomavirus are three pathogens associated with various human cancers. This study aimed to investigate the role of these pathogens in gastric cancer in Moroccan population

Methods: For this, a retrospective study has been conducted on participants attending the gastroenterology department of Hassan II University Hospital of Fez. A total of 279 participants were enrolled. *H. pylori*, *EBV* and *HPV* were detected and genotyped by PCR.

Results: A significant association has been established between *H. pylori*, *EBV* and gastric cancer. 93.4% and 43.3% of gastric cancer cases are related to *H. pylori* and *EBV* respectively ($p \leq 0.01$). *H. pylori*-*EBV* co-infection is responsible of 31.6% of gastric cancer cases ($p < 0.01$). Correlation between pathogens genotypes and gastric cancer shows 55.6% of GC *EBV* positives are carrying the 30bp deletion in *LMP1* gene, while 16% of gastric cancers cases are carrying high-risk genotypes of *HPV* ($p = 0.21$).

Conclusion: The obtained results highlight the possible role of co-infection in gastric cancer development.

1. Introduction

Cancer is a complex and multifactorial disease related to genetic, environmental, lifestyle factors and sometimes infectious agents [1–3]. In fact, the discovery of infectious agents-related to carcinogenesis helped to develop cancer prevention strategies based on vaccination, infection prevention and earlier diagnosis before cell transformation.

Gastric cancer is the fifth most frequently diagnosed cancer and the third leading cause of cancer-related mortality in the world [4]. Its association with *Helicobacter pylori* (*H. pylori*) has been well documented and this bacteria is responsible for a six fold increased risk for gastric non-cardia cancer [5, 6]. Like other cancer-associated infectious agents, *H. pylori* is widespread and harbored by around 75% of people aged 40 years old and above. However, not all carriers will develop gastric cancer [7], thus other factors are required for cell transformation [8]. Among them, carcinogenic viruses could be with major importance. In fact, in the last years, the focus has shifted to the role of *Epstein-Barr virus* (EBV) on GC, and recent studies identified that relatively 10% of patients with gastric cancer exhibited EBV infection [9, 10]. Thus, EBV antigenic epitopes have been detected specifically in gastric cancer cells, demonstrating that EBV may be a virulence factor in gastric cancer [11]. This data was considered by the Cancer Genome Atlas in the novel molecular pathological classification of gastric cancer and EBV-associated gastric cancer (EBVaGC) was categorized as a distinct type of gastric cancer [9]. However, only few studies were interested on and documented the simultaneous presence of EBV and *H. pylori* in gastric cancer specimens. The determined co-infection rate range from 6–12% and the significance of this co-infection was assessed in few meta-analysis [12–14]. Also, EBV and *H. pylori* co-infection with the human immunodeficiency virus (*HIV*) were found to be associated to about 10% of gastric cancer and increased risk of this cancer cases respectively [15–17]. In addition to *EBV* and *HIV*, the implication of *Human*

Papillomavirus (HPV) in gastric cancer development has been recently reported [18] despite the non-conclusive results of several studies [19, 20]. In Morocco, even if the association of *H. pylori* and gastric cancer was well documented, no data on the occurrence and implication of EBV or HPV in gastric cancer cases is available.

The aim of the present study were i) to determine the prevalence of *EBV* and *HPV* and the rate of their co-infection with *H. pylori* in gastric cancer; ii) to correlate their co-presence with gastric cancer in the studied population, iii) to study the correlation between genotypes of infectious pathogens and gastric cancer.

2. Methods

From May 2009 to May 2014, 1134 patients aged 18 years and more, attending Gastroenterology Department of Hassan II University Hospital (CHU) of Fez and undergoing endoscopy for diagnosis of abdominal pain or discomfort were enrolled in a prospective study to determine the *H. pylori* association with gastric pathologies. Patients aged below 18 years or who were on medications (antibiotics, proton pump inhibitors) for the last 3 months, as well as pregnant or nursing women were excluded from this study. Consenting participants had a personal interview to fill a questionnaire before undergoing endoscopy. For illiterate or semi-literate participants, the written consent was read to them by the principal investigator. This study was approved by the CHU Hassan II Ethical Committee.

From the 1134 participants initially enrolled [21], 279 samples were selected to be included in the present study. The selection criteria were based on histological and endoscopic results. Hence, all samples of patients with gastric cancer (GC) and precancerous lesions (pk lesions: metaplasia and /or dysplasia) were used as cases and samples of patients with duodenal ulcer (DU) (only cases whose histological exam didn't show cancerous or pk lesions) were used as control group. Gastritis cases were not included in this study as their evolution to GC or DU still possible. The GC and pk lesions were classified according to the Sydney system [22].

The DNA directly extracted from gastric biopsies was used for *H. pylori*, *EBV* and *HPV* molecular diagnosis using polymerase chain reaction. For all reactions, positive and negative controls were included. The PCR products were resolved in a 2% agarose gel, stained with ethidium bromide and visualized under UV source.

2.1 *H. pylori* molecular diagnosis and genotyping

All specimens have already been analyzed as a part of a study previously conducted by our team [21, 23]. Briefly, PCR using *glmM* primers to detect *H. pylori* as previously described [24] and positive specimens for *H. pylori* were genotyped to determine *CagA* status and *vacA* subtypes by multiplex PCR using specific primers and conditions previously described [25, 26].

2.2 EBV molecular diagnosis and genotyping

The specimens previously analyzed for *H. pylori* diagnosis have been subjected to PCR using TC60 primers to detect *BamW* gene of *EBV* as previously described [27] and *LMP1* genes was characterized for all EBV positive specimens using specific primers [28, 29].

2.3 HPV molecular diagnosis and genotyping

HPV DNA was detected by PCR using the primers GP5+/GP6+ as previously described [30]. HPV positive samples were then analyzed to identify the low and high-risk genotypes and to determine the specific high-risk genotypes using a recently developed genotyping method. In fact. Two separate multiplex PCR were setup for the specific identification of 16 high risk-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 73 and 82).

2.4 Statistical analysis

All collected data were entered on an Excel spread sheet; statistical analysis was then performed using SPSS (Statistical product and services solutions, version20, SPSS Inc. Chicago, Illinois, USA) software. The studied population was described and then the association's pathogens-histological lesions and pathogens genotypes-histological lesions were studied. Chi-square or Fisher's exact tests were applied to establish all statistically significant associations. All Chi2 test results with p-values ≤ 0.05 were considered as statistically significant.

3. Results

A total of 279 consenting participants were enrolled in this study. They were 188 (67.4%) men and 91 (32.6%) women with an average age of 52 years, ranging from 18 to 90 years, and only 88/272 (32.4%) and 42/231 (15.4%) of them are smokers and alcoholics, respectively. Most of participants (124/279) were with pk lesions and 76 were with GC. In this study, DU cases (79/279) were considered as control group.

Molecular diagnosis was performed to determine the prevalence of *H. pylori*, HPV and *EBV* infections on the studied population. The results confirm the presence of *H. pylori*, *EBV* and HPV infection in 57.3% (160/279), 28% (78/279) and 26.2% (73/279) cases respectively.

H. pylori genotyping shows that *cagA* positive, *VacA s1m1*, *vacA s2m1*, *vacA s1m2* and *vacA s2m2* strains were detected in 68.9%, 42.7%, 12.6%, 10.7% and 34% cases respectively.

EBV positive samples have been genotyped and 62.8% of cases carried the 30bp *LMP1* deletion (versus 12.8% wild type).

HPV positives samples genotyping revealed that 87.7% cases were with low risk strains; while 12.3% were with high risk ones, which correspond to HPV 35, 45, 51 and 53. HPV 53 was the most prevalent genotype in the high risk strains.

3.1 Correlation between infection and the studied groups

Infections status has been correlated to the pathologic groups in this series and the results show a significant association between *H. pylori*, EBV infections and GC ($p \leq 0.01$) separately. In fact, 93.4% and 43.4% of GC cases were related to *H. pylori* and EBV infection respectively, while no association has been obtained with HPV infection (Table 1).

Table 1
Correlation between infection status and studied groups

	GC n(%)	pk lesions n(%)	Duodenal ulcer n(%)	p value
H. pylori	71/76 (93.4)	49/124 (39.5)	40/79 (50.6)	0.0000
Positive				
Negative	5/76 (6.6)	75/124 (60.5)	39/79 (49.4)	
EBV	33/76 (43.4)	24/124 (19.4)	21/79 (26.6)	0.0011
Positive				
Negative	43/76 (56.6)	100/124 (80.6)	58/79 (73.4)	
HPV	24/76 (31.6)	31/124 (25)	18/79 (22.8)	0.42
Positive				
Negative	52/76 (68.4)	93/124 (75)	61/79 (77.2)	
EBV: <i>Epstein-Barr virus</i> ; GC: gastric cancer; HPV: <i>Human Papillomavirus</i> ; pk lesions: precancerous lesions				

The *H. pylori* co-infection with EBV and HPV were correlated with the gastric pathologies. The obtained results show that third (31.6%) of GC cases are associated to *H. pylori*-EBV co-infection while 34.2% of DU cases were significantly associated to *H. pylori* single infection (Table 2) and 33% of Pk lesions cases didn't show an amplification of any of those micro-organisms.

Table 2
H. pylori, EBV and HPV co-infection correlated to the studied groups

	GC n(%)	Pk lesions n(%)	Duodenal Ulcer n(%)	p-value
Single infection				0.0000
H. pylori	23/76(30.3)	34/124(27.4)	27/79(34.2)	
HPV	0/76(0)	19/124(15.3)	10/79(12.7)	
EBV	1/76(1.3)	11/124(8.9)	8/79(10.1)	
Co-infection				
H. pylori - EBV- HPV	8/76(10.5)	2/124(1.6)	2/79(2.5)	
H. pylori - EBV	24/76(31.6)	7/124(5.6)	8/79(10.1)	
H. pylori - HPV	16/76(21.1)	6/124(4.8)	3/79(3.8)	
EBV - HPV	0/76(0)	4/124(3.2)	3/79(3.8)	
No infection	4/76(5.3)	41/124(33.1)	18/79(22.8)	
EBV: <i>Epstein-Barr virus</i> ; GC: gastric cancer; HPV: <i>Human Papillomavirus</i> ; pk lesions: precancerous lesions				

Also, the correlation between participant's gender and EBV infection status according to studied groups show that 59.3% of EBV infected women have GC (p= 0.03) (Table 3).

Table 3
 Correlation of gender with EBV infection in the studied groups

		<i>EBV +</i>	<i>EBV-</i>	p-value
GC	Gender <i>Woman</i>	16/27 (59.3%)	11/27 (40.7%)	0.03
	<i>Man</i>	17/49 (34.7%)	32/49 (65.3%)	
Pk lesions	Gender <i>Woman</i>	7/45 (15.6%)	38/45 (84.4%)	0.41
	<i>Man</i>	17/79 (21.5%)	62/79(78.5%)	
Duodenal Ulcer	Gender <i>Woman</i>	5/19 (26.3%)	14/19 (73.7%)	0.97
	<i>Man</i>	16/60 (26.7%)	44/60 (73.3%)	
EBV: <i>Epstein-Barr virus</i> ; GC: gastric cancer; HPV: <i>Human Papillomavirus</i> ; pk lesions: precancerous lesions				

3.2 Correlation of pathogens genotypes and gastric pathologies

The correlation between *H. pylori*, EBV and HPV genotypes and pathologic groups has been studied. *VacAs1m1 H. pylori* genotype was the most predominant in GC cases [45.3% versus 42.3% in pk lesions and 37.5% in DU group] ($p=0.01$). However, no significant association was obtained between EBV or HPV genotypes and gastric pathologies. Nevertheless, *EBV* LMP wild type is more predominant in GC cases and 16% of HPV positive GC cases are carrying high-risk genotypes versus 0% of DU cases (Table 4).

Table 4
H. pylori, EBV and HPV genotypes correlated to the studied groups

		<i>GC</i>	<i>Pk lesions</i>	<i>Duodenal Ulcer</i>	p-value
H. pylori genotypes	VacAs1m1	24/53 (45.3%)	11/26 (42.3%)	9/24 (37.5%)	0.01
	VacAs2m1	11/53 (20.8%)	0/26 (0%)	2/24 (8.3%)	
	VacAs1m2	2/53 (3.8%)	3/26 (11.5%)	6/24 (25%)	
	VacAs2m2	16/53(30.2%)	12/26(46.2%)	7/24(29.2%)	
	CagA Negative	19/53 (35.8%)	8/26 (30.7%)	5/24 (20.8%)	0.41
	Positive	34/53 (64.2%)	18/26 (69.2%)	19/24 (79.2%)	
	LMP1 wild type	7/33 (21.2%)	2/24 (8.3%)	1/21 (4.8%)	0.24
	30pb deletion	18/33 (54.5%)	18/24 (75%)	13/21(61.9%)	
	No amplification	8/33 (24.2%)	4/24 (16.7%)	7/21 (33.3%)	
HPV genotypes	Low risk	21/25 (84%)	26/31 (83.9%)	17/17 (100%)	0.21
	High risk	4/25 (16%)	5/31 (16.1%)	0/17 (0%)	
HR-HPV genotypes	HPV 35	0	1 (20%)	0	0.41
	HPV 45	1 (25%)	0	0	
	HPV 53	3 (75%)	3 (60%)	0	
	HPV 51/53	0	1 (20%)	0	
<i>cagA</i> : cytotoxin-associated gene; EBV: <i>Epstein-Barr virus</i> ; GC: gastric cancer; HPV: <i>Human Papillomavirus</i> ; pk lesions: precancerous lesions; <i>vacA</i> : vacuolating cytotoxin, HR-HPV <i>genotypes</i> : <i>High risk Human Papillomavirus</i> genotypes					

A significant association was obtained between *H. pylori cagA* status and EBV infection in Pk lesions. Thus, 62.5% of *H.pylori* cases empty for *cagA* (*cagA*⁻) were EBV co-infected (p= 0.04) (Table 5).

Table 5
Correlation of *cagA* status with EBV infection in the studied groups

		<i>EBV</i> +	<i>EBV</i> -	p value
GC	<i>cagA</i> Negative	9/19 (47.4%)	10/19 (52.6%)	0.81
	<i>Positive</i>	15/34 (44.1%)	19/34 (55.9%)	
Pk lesions	<i>cagA</i> Negative	5/8 (62.5%)	3/8 (37.5%)	0.04
	<i>Positive</i>	4/18 (22.2%)	14/18 (77.8%)	
Duodenal Ulcer	<i>cagA</i> Negative	2/5 (40%)	3/5 (60%)	0.23
	<i>Positive</i>	3/19 (15.8%)	16/19 (84.2%)	

4. Discussion

H. pylori infection is strongly associated to GC development which is one of the most common cancers, contributing to 6.8% of total cancer cases and 8.8% of total cancer-associated mortality worldwide [31–35]. In a previous study, conducted by our team, in the central north region of Morocco [36], we came to the evidence that all our GC cases are not *H. pylori* dependent. In fact, recent studies reported the implication of other factors in the gastric carcinogenesis. *EBV* and HPV has been described as potentially responsible of some of these cases [7, 15, 18, 37] and EBV-associated gastric cancer (EBVaGC) was categorized as a distinct type of gastric cancer according to the last Cancer Genome Atlas classification.

To date, *H. pylori* co-infection with *EBV* and especially with HPV remains little explored in GC [18, 38–40], and most of conducted study studies on this field were concentrated in Latin America except one that were conducted in India. In our knowledge, this is the first study conducted in Morocco and North Africa with the aims to investigate the implication of these pathogens and their co-infection with *H. pylori* mainly in GC cases and to determine the genotypes implicated in the gastric carcinogenesis. This will help to understand GC causes and establish adapted preventing strategy.

In this study, the prevalence of *H. pylori*, *EBV* and HPV infection in all the studied groups, namely GC, pk lesions and DU groups was determined. The choice of the two first groups was based on the histological lesions evolution, while DU is considered as control group. Patients with DU were used as control group because gastritis can evolved towards pk lesions, which predispose to GC disease, besides DU and GC seem to be mutually exclusive outcomes of *H. pylori* infection [41].

The obtained rates of *H. pylori*, *EBV* and HPV infections were 57.3%, 28% and 26.2% respectively. These rates increase clearly in GC cases to reach 93.4%, ($p < 0.01$) for *H. pylori*, 43.4% for *EBV* ($p = 0.01$) and 31.6% for HPV ($p = 0.42$). The prevalence of *H. pylori* infection in GC corroborate our previous results [42] and the results reported in north Brazil where *H. pylori* infection has been detected in 88% of GC cases, while *EBV* infection has only been detected in 9.6% of GC cases which is lower than the rate detected in

our population [43]. The studied population and the EBV diagnosis methods can explain this difference. In fact, in this Brazilian study the RNA in situ hybridization was used for EBV diagnosis. Also, the HPV prevalence detected in our GC group (31.6%) is comparable to that recently reported in a meta-analysis conducted in Asia (28%) [18].

The *H. pylori*-EBV or HPV co-infection has been correlated to the studied groups. These correlations show that most GC cases are associated to the *H. pylori*-EBV co-infection (31.6%) compared to the control group (10.1%). *H. pylori* single infection and the *H. pylori*-HPV co-infection are responsible of 30.3% and 21.1% of GC cases respectively ($p < 0.01$). These significant associations confirm that *H. pylori*-EBV co-infections are risk factors for GC development. However, the fact that no cases of HPV single infection was observed in GC cases while 21.1% of GC cases presented co-infection versus 3,8 in control group leads to suppose that HPV may be a cofactor of GC development in *H. pylori* positive patients. Also, it's interesting to highlight that no case of GC has been related to HPV-EBV co-infection and that 5.3% of our GC cases are pathogens independent ($p < 0.01$). In these GC cases, other risk factors can be incriminated such as nutritional, environmental and genetic predisposition. This also can be explained by the fact that *H. pylori* can likely disappear spontaneously during malignant transformation of gastric epithelia as reported by several studies [44, 45]. The *H. pylori* co-infection with EBV and HPV in the studied groups has also been investigated according to the participants age (younger and older than 40 years). Despite the non-significance of the association in all cases, it's clear that GC rate increase highly in case of *H. pylori*-EBV and/or HPV co-infection. In fact, in group 1, only 29.2%, 20% and 0% of participants *H. pylori*+, EBV+ and HPV+ respectively have GC compared to 33.3% in case of *H. pylori*-EBV co-infection and 60% in case of *H. pylori*-HPV co-infection. While in group 2 *H. pylori*+, EBV+ and HPV+ participants, GC rate is 26.7%, 0% and 0% respectively, this rate increase clearly in case of *H. pylori*-EBV (66.7%) and *H. pylori*-HPV (65%) co-infections. These tendencies show that *H. pylori* co-infection with EBV and/or HPV is a risk factor for GC development and that this risk increases with age.

As *H. pylori* infection is the main cause of GC development, almost all *H. pylori* genes have been widely studied and the most virulent genotypes identified. In our study, the results show that 45.3% of GC cases are *VacA s1m1* ($p = 0.01$) and 64.2% are *cagA* positive ($p = 0.41$). The rate of *cagA* is comparable to that reported in north Brazil (67.2%) [43]. Therefore, EBV and HPV genes have been well studied and identified mostly in nasopharyngeal carcinoma and cervical cancer respectively but limited in GC. For this reason, we proposed to investigate the correlation between EBV and HPV genotypes with the studied groups. The 30-bp *LMP1* gene deletion was observed in 54.5% of our EBV+ GC cases that is comparable to the results of Brazilian study (50%) but lower than that of Japanese study (88.5% and 91.7%) [46, 47].

In our study, women are more likely than men to develop EBV-associated gastric cancer. In fact, a significant association was found between gender and EBV infection in GC cases. Thus, 59% of women with GC were EBV positives vs 34% of man. However, 51.5% of EBV + GC+ were man. These data corroborate the data of meta-analysis conducted by Tavakoli et al. on 2020 [48]. If *H. pylori* infection and epigenetic conditions resulting on the infection play role in EBV reactivation, this reactivation in women is more pronounced which can be related to factors such as to hormonal ones.

H. pylori cagA is more co-present with EBV. This tendency is observed in all cases but is significant in Pk lesion cases. Supposing that *H. pylori* play role in EBV reactivation, the last data lets suggest that some *H. pylori* strains and specially Cag- strains are more likely applied in this reactivation.

The implication of HPV in GC has long been controversial [19, 37, 49]. When a recent Brazilian study reported a lower prevalence of HPV in GC cases (3%) [50], a meta-analysis conducted on 1917 cases reported a higher rates (a pooled prevalence : 28%) and that HPV prevalence in GC cases is 7 fold higher than in normal gastric tissues [18]. In our series, 31.6% of GC cases are HPV infected which is concordant with the results of the meta-analysis. The genotyping analysis shows that only 16% of GC-HPV positives cases were with high-risks HPV vs 0% in DU cases (p=0.21). However, only the HPV 16 and 18 strains were detected in Brazilian GC cases [50]. The obtained difference can be explained by geographical HPV distribution and environmental and/or lifestyle factors. Even if those associations are not significant, it will be interesting to define if the co-infection constitutes an accumulated risk to develop GC or if it's a result of disease development that lead to host fragility and help a viral reactivation or super infection. For that, further studies on a larger population are needed.

This study has some limitations. The first limitation is the small number of participants and specifically in GC patient with HPV single infection or EBV single infection, which makes difficult to evaluate role of EBV or HPV in gastric carcinogenesis. Secondly, the histological classification of gastric cancer was not based on Lauren's criteria in which intestinal type and diffuse type adenocarcinoma are the two major histologic subtypes, because the distribution of EBV is different depending on intestinal or diffuse type [51]. Even if a recent meta-analysis showed that the prevalence of EBV was similar in intestinal and diffuse types, and no significant association was found between EBV infection and the histological type [48]

5. Conclusion

The obtained results show that *H. pylori* and EBV infections are significantly associated to GC and that the majority of GC cases are associated to *H. pylori*-EBV co-infection. The most recognized virulent genotypes of different pathogens seem to be those associated to gastric cancer. So, participants with co-infection and /or carrying the most virulent genotypes may need more attention to prevent gastric cancer development.

Declarations

Funding:

This work was supported by the National Centre for Scientific and Technical Research (CNRST) and the "Ministère de l'éducation nationale, de la formation professionnelle, de l'enseignement supérieur et de la recherche scientifique" Morocco (Ministry of National Education, Vocational Training, Higher Education and Scientific Research) [grant number: 03P/2016]; the University Hospital Hassan II of Fez [grant

number: PR04-12 13] and Laboratory of Human Pathology Biomedicine and Environment of the Faculty of Medicine and Pharmacy of Fez

Conflicts of interest:

None

Ethics approval and consent to participate:

This study was approved, on April 22, 2009, by the Institutional Review Board of the Hassan II University Hospital of Fez, Morocco with the ethical permission number (03AV/2009). All subjects included in the study provide his/her written informed consent on study participation.

Availability of data:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions:

All authors contributed equally to this work

Acknowledgments:

We would like to express our deep gratitude to all funders and thank the staffs of the gastroenterology unit and anatomo-pathology department for their help in the collection and preparation of specimens.

References

1. Amieva M, Peek RM. Pathobiology of *Helicobacter pylori*-Induced Gastric Cancer. *Gastroenterology*. W.B. Saunders; 2016. p. 64–78.
2. Gurzu S, Jung I, Orlowska J, Sugimura H, Kadar Z, Turdean S, et al. Hereditary diffuse gastric cancer - An overview. *Pathol. Res. Pract.* Elsevier GmbH; 2015. p. 629–32.
3. Kim J, Cho YA, Choi WJ, Jeong SH. Gene-diet interactions in gastric cancer risk: A systematic review. *World J. Gastroenterol.* WJG Press; 2014. p. 9600–10.
4. 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* [Internet]. 2018 [cited 2019 Dec 5];68:394–424. Available from: <http://doi.wiley.com/10.3322/caac.21492>
5. Kusters JG, van Vliet AHM, Kuipers EJ. Pathogenesis of *Helicobacter pylori* {Infection}. *Clin Microbiol Rev* [Internet]. 2006 [cited 2016 May 14];19:449–90. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1539101/>

6. Webb PM, Law M, Varghese C, Forman D. Gastric cancer and *Helicobacter pylori*: A combined analysis of 12 case control studies nested within prospective cohorts. *Gut*. 2001;49:347–53.
7. Fukayama M, Ushiku T. Epstein-Barr virus-associated gastric carcinoma. *Pathol. Res. Pract. Urban & Fischer*; 2011. p. 529–37.
8. Figura N. *Helicobacter pylori* infection and gastric carcinoma: Not all the strains and patients are alike . *World J Gastrointest Oncol*. Baishideng Publishing Group Inc.; 2016;8:40.
9. Camargo MC, Bowlby R, Chu A, Pedamallu CS, Thorsson V, Elmore S, et al. Validation and calibration of next-generation sequencing to identify Epstein-Barr virus-positive gastric cancer in The Cancer Genome Atlas. *Gastric Cancer*. Springer Tokyo; 2016;19:676–81.
10. Camargo MC, Kim KM, Matsuo K, Torres J, Liao LM, Morgan DR, et al. Anti-*Helicobacter pylori* Antibody Profiles in Epstein-Barr virus (EBV)-Positive and EBV-Negative Gastric Cancer. *Helicobacter*. Blackwell Publishing Ltd; 2016;21:153–7.
11. Liu Y, Yang W, Pan Y, Ji J, Lu Z, Ke Y. Genome-wide analysis of Epstein-Barr virus (EBV) isolated from EBV-associated gastric carcinoma (EBVaGC). *Oncotarget*. Impact Journals LLC; 2016;7:4903–14.
12. Martínez-López JLE, Torres J, Camorlinga-Ponce M, Mantilla A, Leal YA, Fuentes-Pananá EM. Evidence of Epstein-Barr virus association with gastric cancer and non-atrophic gastritis. *Viruses*. Multidisciplinary Digital Publishing Institute (MDPI); 2014;6:301–18.
13. Zong L, Seto Y. CpG Island Methylator Phenotype, *Helicobacter pylori*, Epstein-Barr Virus, and Microsatellite Instability and Prognosis in Gastric Cancer: A Systematic Review and Meta-Analysis. Boneca IG, editor. *PLoS One* [Internet]. Public Library of Science; 2014 [cited 2020 Oct 9];9:e86097. Available from: <https://dx.plos.org/10.1371/journal.pone.0086097>
14. Kim JY, Bae BN, Kang G, Kim HJ, Park K. Cytokine expression associated with *Helicobacter pylori* and Epstein-Barr virus infection in gastric carcinogenesis. *APMIS*. Blackwell Munksgaard; 2017;125:808–15.
15. Liang Q, Yao X, Tang S, Zhang J, Yau TO, Li X, et al. Integrative identification of Epstein-Barr virus-associated mutations and epigenetic alterations in gastric cancer. *Gastroenterology*. W.B. Saunders; 2014;147:1350-1362.e4.
16. Villain P, Gonzalez P, Almonte M, Franceschi S, Dillner J, Anttila A, et al. European Code against Cancer 4th Edition: Infections and Cancer. *Cancer Epidemiol*. Elsevier Ltd; 2015;39:S120–38.
17. Agalliu I, Gapstur S, Chen Z, Wang T, Anderson RL, Teras L, et al. Associations of oral α -, β -, and γ -human papillomavirus types with risk of incident head and neck cancer. *JAMA Oncol*. American Medical Association; 2016;2:599–606.
18. 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. *Onco Targets Ther*. Dove Medical Press Ltd.; 2016;9:7105–14.
19. Koshiol J, Wei WQ, Kreimer AR, Ren JS, Gravitt P, Chen W, et al. The gastric cardia is not a target for human papillomavirus-induced carcinogenesis. *Cancer Epidemiol Biomarkers Prev*. *Cancer Epidemiol Biomarkers Prev*; 2010;19:1137–9.

20. 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;36:183–4.
21. El Khadir M, Alaoui Boukhris S, Benajah D-A, El Rhazi K, Ibrahimi SA, El Abkari M, et al. VacA and CagA Status as Biomarker of Two Opposite End Outcomes of *Helicobacter pylori* Infection (Gastric Cancer and Duodenal Ulcer) in a Moroccan Population. Soutto M, editor. *PLoS One* [Internet]. Public Library of Science; 2017 [cited 2017 Sep 12];12:e0170616. Available from: <http://dx.plos.org/10.1371/journal.pone.0170616>
22. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. {The} updated {Sydney} {System}. {International} {Workshop} on the {Histopathology} of {Gastritis}, {Houston} 1994. *Am J Surg Pathol.* 1996;20:1161–81.
23. Alaoui Boukhris S, Benajah D -a., Rhazi K, Ibrahimi SA, Nejari C, Amarti A, et al. Erratum to: {Prevalence} and distribution of {*Helicobacter*} *pylori* {cagA} and {vacA} genotypes in the {Moroccan} population with gastric disease. *Eur J Clin Microbiol Infect Dis* [Internet]. 2013 [cited 2016 Aug 10];32:969. Available from: <http://link.springer.com/10.1007/s10096-013-1887-8>
24. Lage AP, Godfroid E, Fauconnier A, Burette A, Butzler JP, Bollen A, et al. Diagnosis of {*Helicobacter*} *pylori* infection by {PCR}: comparison with other invasive techniques and detection of {cagA} gene in gastric biopsy specimens. *J Clin Microbiol* [Internet]. 1995 [cited 2016 Aug 10];33:2752–6. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC228568/>
25. Chattopadhyay S, Patra R, Ramamurthy T, Chowdhury A, Santra A, Dhali GK, et al. Multiplex {PCR} {Assay} for {Rapid} {Detection} and {Genotyping} of {*Helicobacter*} *pylori* {Directly} from {Biopsy} {Specimens}. *J Clin Microbiol* [Internet]. 2004 [cited 2016 Aug 10];42:2821–4. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC427847/>
26. Bolek BK, Salih BA, Sander E. Genotyping of {*Helicobacter*} *pylori* strains from gastric biopsies by multiplex polymerase chain reaction. {How} advantageous is it? *Diagn Microbiol Infect Dis* [Internet]. 2007 [cited 2016 Jun 19];58:67–70. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0732889306004846>
27. Saito I, Servenius B, Compton T, Fox RI. Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjogren's syndrome. *J Exp Med.* 1989;169:2191–8.
28. Lung ML, Chang GC. Detection of distinct Epstein-Barr virus genotypes in NPC biopsies from Southern Chinese and Caucasians. *Int J Cancer* [Internet]. John Wiley & Sons, Ltd; 1992 [cited 2020 Oct 8];52:34–7. Available from: <http://doi.wiley.com/10.1002/ijc.2910520108>
29. Lin S xia, Zong Y sheng, Zhang M, Han A jia, Zhong B ling, Liang Y jie. Study of sequence variations of Epstein-Barr virus LMP1 gene in nasopharyngeal carcinoma. *Zhonghua Bing Li Xue Za Zhi.* 2005;34:791–5.
30. de Roda Husman AM, Snijders PJF, Stel H V., van den Brule AJC, Meijer CJLM, Walboomers JMM. Processing of long-stored archival cervical smears for human papillomavirus detection by the

- polymerase chain reaction. Br J Cancer. Br J Cancer; 1995;72:412–7.
31. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: {Sources}, methods and major patterns in {GLOBOCAN} 2012: {Globocan} 2012. Int J Cancer [Internet]. 2015 [cited 2016 Sep 20];136:E359–E386. Available from: <http://doi.wiley.com/10.1002/ijc.29210>
 32. Ishaq S, Nunn L. Helicobacter pylori and gastric cancer: A state of the art review. Gastroenterol. Hepatol. from Bed to Bench. Research Institute for Gastroenterology and Liver Diseases; 2015. p. S6–14.
 33. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. Wiley; 2011;61:69–90.
 34. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravalle G, Dammacco F. H. pylori infection and gastric cancer: {State} of the art ({Review}). Int J Oncol [Internet]. 2013 [cited 2017 Aug 22];42:5–18. Available from: <http://www.spandidos-publications.com/ijo/42/1/5/abstract>
 35. Globocan 2012 - {Home} [Internet]. [cited 2016 Nov 21]. Available from: <http://globocan.iarc.fr/Default.aspx>
 36. Alaoui Boukhris S, Amarti A, El Rhazi K, El Khadir M, Benajah D-A, Ibrahimi SA, et al. Helicobacter pylori genotypes associated with gastric histo-pathological damages in a {Moroccan} population. PLoS One. 2013;8:e82646.
 37. Snietura M, Waniczek D, Piglowski W, Kopec A, Nowakowska-Zajdel E, Lorenc Z, et al. Potential role of human papilloma virus in the pathogenesis of gastric cancer. World J Gastroenterol. WJG Press; 2014;20:6632–7.
 38. Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. Am J Pathol. American Society for Investigative Pathology; 1992;140:769–74.
 39. Abe H, Kaneda A, Fukayama M. Epstein-Barr virus-associated gastric carcinoma: Use of host cell machineries and somatic gene mutations. Pathobiology. S. Karger AG; 2015. p. 212–3.
 40. Shinozaki-Ushiku A, Kunita A, Fukayama M. Update on epstein-barr virus and gastric cancer (review). Int. J. Oncol. Spandidos Publications; 2015. p. 1421–34.
 41. Hansson LE, Nyrén O, Hsing AW, Bergström R, Josefsson S, Chow WH, et al. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med. 1996;335:242–9.
 42. El Khadir M, Alaoui Boukhris S, Benajah D, Ibrahimi SA, Chbani L, El Abkari M, et al. <scp> VacA </scp> genotypes and <scp>cagA-EPIYA-C</scp> motifs of *Helicobacter pylori* and gastric histopathological lesions. Int J Cancer [Internet]. John Wiley & Sons, Ltd; 2020 [cited 2020 Jun 28];ijc.33158. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.33158>
 43. de Souza CRT, de Oliveira KS, Ferraz JJS, Leal MF, Calcagno DQ, Seabra AD, et al. Occurrence of Helicobacter pylori and Epstein-Barr virus infection in endoscopic and gastric cancer patients from Northern Brazil. BMC Gastroenterol. BioMed Central Ltd.; 2014;14.
 44. Kokkola A, Kosunen TU, Puolakkainen P, Sipponen P, Härkönen M, Laxén F, et al. Spontaneous disappearance of Helicobacter pylori antibodies in patients with advanced atrophic corpus gastritis.

APMIS. John Wiley & Sons, Ltd; 2003;111:619–24.

45. Perri F, Pastore M, Clemente R, Festa V, Quitadamo M, Niro G, et al. Helicobacter pylori infection may undergo spontaneous eradication in children: A 2-year follow-up study. J Pediatr Gastroenterol Nutr. J Pediatr Gastroenterol Nutr; 1998;27:181–3.
46. Hayashi K, Chen WG, Chen YY, Murakami I, Chen HL, Ohara N, et al. Deletion of Epstein-Barr virus latent membrane protein 1 gene in Japanese and Brazilian gastric carcinomas, metastatic lesions, and reactive lymphocytes. Am J Pathol. American Society for Investigative Pathology; 1998;152:191–8.
47. Mori S, Itoh T, Tokunaga M, Eizuru Y. Deletions and single-base mutations within the carboxy-terminal region of the latent membrane protein 1 oncogene in Epstein-Barr virus-related gastric cancers of southern Japan. J Med Virol. J Med Virol; 1999;57:152–8.
48. Tavakoli A, Monavari SH, Solaymani Mohammadi F, Kiani SJ, Armat S, Farahmand M. Association between Epstein-Barr virus infection and gastric cancer: A systematic review and meta-analysis [Internet]. BMC Cancer. BioMed Central Ltd.; 2020 [cited 2020 Oct 9]. p. 493. Available from: <https://bmccancer.biomedcentral.com/articles/10.1186/s12885-020-07013-x>
49. Xu W guo, Zhang L jian, Lu Z ming, Li J you, Ke Y, Xu G wei. Detection of human papillomavirus type 16 E6 mRNA in carcinomas of upper digestive tract. Zhonghua Yi Xue Za Zhi. 2003;83:1910–4.
50. 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. Baishideng Publishing Group Co., Limited; 2018;24:4928–38.
51. Wu MS, Shun CT, Wu CC, Hsu TY, Lin MT, Chang MC, et al. Epstein-Barr virus - Associated gastric carcinomas: Relation to H. pylori infection and genetic alterations. Gastroenterology. W.B. Saunders; 2000;118:1031–8.