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Characteristics of gastric carcinoma associated with Epstein Barr virus in Algeria

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ABSTRACT

Epstein-Barr virus is associated with gastric carcinoma. There has been no study about the frequency of Epstein Barr virus associated gastric carcinoma (EBVaGC) in Algeria an endemic area of nasopharyngeal carcinoma. The objective of this study is to investigate the prevalence of EBVaGC among Algerian patients and to define EBV latency patterns in this tumor.The expression of latent membrane protein-1 (LMP-1) was evaluated in EBV positive cases with specific monoclonal antibodies using immunofluorescence and immunohistochemistry techniques.97 cases selected of gastric carcinoma were examined for EBV infection using fluorescence in situ hybridization assay to detect EBV-encoded small RNAs (EBER-1 and EBER-2). Combined immunofluorescence and fluorescence in situ hybridization was carried out in 15 gastric carcinoma cases to confirm the presence of EBERs and LMP-1 simultaneously. We have shown that 22 out of 97 cases (22.7%) expressed EBV. EBERs were present within the malignant epithelial cells and in precancerous lesions showing dysplasia tissue, but in none of non-neoplastic cases. In addition, LMP-1 was expressed in 8 of 22 EBV-positive simples. The expression was membranous/cytoplasmic, restricted to the malignant cells and absent in non-neoplastic tissues. Combined immunofluorescence and fluorescence in situ hybridization determined a colocalisation of EBERs and LMP-1 in gastric carcinoma cells. We conclude that the prevalence of EBVaGC is 22.7 % in Algeria with specific clinicopathologic features, and the type of latency is similar to the type of latency of nasopharyngeal carcinoma.

Keywords: Epstein Barr virus,, gastric carcinoma,, EBV-encoded small RNAs,, latent membrane proteins-1.,

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INTRODUCTION

The Epstein Barr Virus (EBV)is a ubiquitous herpes virus infecting 95 % of world's population. EBV was the first virus shown to contribute to major cell proliferation disorders in humans; it is associated with wide variety of malignancies including Burkitt lymphoma[1],Hodgkin's disease[2],post-transplant lymphoproliferative disorder, nasopharyngeal carcinoma (NPC) and gastric carcinoma (GC)[1];[2].They first detected EBV infection inundifferentiated lymphoepithelioma-like carcinoma (LELC) by polymerase chain reaction (PCR), it is an uncommon type characterized by marked stromal lymphocytic infiltration[3]. Subsequently, further investigation determined that EBV may be present in typical gastric adenocarcinoma and in adjacent dysplastic epithelia[4].

In contrast to other EBV-associated malignancies, such as endemic Burkitt's lymphoma and nasopharyngeal carcinoma, EBV positive gastric cancer makeup the largest group of EBV-associated malignancies with worldwide geographical variations (5 Takada, 2000).However, there are some regional differences in the incidence of EBV associated GC (EBVaGC), it is distributed from the highest (16-18%) in the USA and Germany to the lowest (4.3%, 3.9%) respectively in China and in Peru([5]; [6];[7]). EBVaGC exhibit some characteristic clinico-pathologic features such as high incidence in (LELC) and remnant gastric carcinoma, a male predominance and localization in the proximal stomach([8]; [9]).It is also related to diffuse histological subtype with abundant infiltrating lymphocytes and poor to moderate differentiation([10]; [6]).

The diagnosis of EBV infection requires not only the detection and localization of the viral genome but also the detection of viral gene products. In situ hybridization and immunostaining covers this point. The two small non-coding EBER-1 and EBER-2 are heavily transcribed and abundant RNAs EBV in latent infection[11].In situ hybridization of EBERs is the gold standard assay to detect EBV latent infection in paraffin-embedded tissue sections[12].Of the EBV proteins, latent membrane protein 1 (LMP-1) has been postulated to be the major oncogenic protein.LMP-1 can transform rodent fibroblast cell lines in nude mice and prevents cells from apoptosis to produce immortalized cells[13].In human B cells, LMP1 is essential for B-lymphocyte transformation and immortalized state[14].

Gastric carcinoma represents the fourth most common type of cancer and the second leading cause of cancer related deathworldwide[16].In Algeria, the stomach cancer is the fifth most prevalent cancer among the Algerian population with the annual incidence of 5.5/100 000 persons[17].Moreover, North Africa is one of the major nasopharyngeal carcinoma endemic regions[18].The incidence of NPC is high in Algeria and it is also for Hodgkin and Burkitt lymphoma[19].Precise information on EBVaGC especially on geographic differences of frequency is limited and there is no study about the prevalence of EBVaGC in Algeria.

The aim of the present study was to determine the frequency of EBV-related gastric carcinoma in cases from Algeria, using fluorescence in situ hybridization(FISH) for the detection of EBERs transcripts. Subsequently, we studied the EBV positive cases by immunohistology techniques with specific monoclonal antibodies for the detection of EBV latent membrane protein-1 (LMP-1).We additionally utilized sequential labeling for LMP-1 by immunofluorescence and EBER by fluorescence in situ hybridization to confirm the possible role of EBV in the pathogenesis of gastric carcinoma.

MATERIALS AND METHODS

Specimens

Formalin fixed-paraffin-embedded blocks from 113 surgically resected gastric specimens obtained from the pathology department of NEDIR Mohammed University Hospital, Tizi-ouzou, Algeria, between 2010 and 2013. This study was approved by the Medical Ethics Committee of the University Hospital of NEDIR Mohammed (Ref: CSF/SBSA/002013) in accordance with the guidelines of the Declaration of Helsinki 1975. For further molecular evaluations, the specimens selected were sending to the institute of anatomy and molecular embryology at Ruhr University Bochum Germany.

Pathologic characteristics

Among 113 cases studied, 97 cases were diagnosed with gastric carcinoma, 6 cases of pre-neoplastic gastric tissue (dysplasia) and 10 cases of non-neoplastic mucosa showing gastritis tissue. The clinical data concerning the age, sex, and anatomic site of tumor were evaluated by reviewing the medical chats and pathological records. Glass slides were reviewed to determine the histological types according to the criteria of [20]as intestinal type and diffuse type, the cases are sub-classified according to the guidelines of the [21]to papillary adenocarcinoma (pap), well differentiated tubular adenocarcinoma (tub1), moderately differentiated tubular adenocarcinoma (tub2), solid type poorly differentiated adenocarcinoma (por1), non-solid type poorly differentiated adenocarcinoma (por2), signet ring cell carcinoma (sig) and special type of poorly differentiated carcinoma (LELC).

PNA probe and antibodies

The probes used in this study are EBER PNA probes Y5200, purchased from Dako. Y5200 is a mixture of 4 different fluorescein-labeled PNA probe complementary to the EBERs RNA molecules (EBER-1 and EBER-2). An oligo (d)T control probes was used served as a control for RNA preservation in histological sections. For the

detection of LMP-1 we have used two different mouse primary monoclonal antibodies: the S12 was kindly provides by Tadamasa Ooka (Laboratoire de virologie moléculaire Lyon France) and the CS1-4 were obtained from Dako. Secondary antibodies used are Alexa Fluor 488 conjugated FITC (green) and Alexa fluor 568 conjugated TRITC (red) (Invitrogen. USA).

Fluorescence in situ hybridization

FISH was carried out for small EBV-encoded RNAs (EBER1-2) in paraffin-embedded tissue sections. Hematoxylin/eosin (HE) staining was done in adjacent section for histological identification of the site of fluorescence. The reaction was performed according to the manufacturer's instruction (PNA ISH detection kit, Dako) with slight modification. In brief, 4µm thick sections cut from paraffin block and mounted on amino-propyl triethoxysilane (sigma chemical) coated slides. The slides were dewaxed with xylene and rehydrated through alcohols to water, the sections were treated with proteinase K diluted with TBS for 30 minutes at 37° to increase their permeability to the probe and then passed in glycin diluted in TBS for 3s to inactivate proteinase K. Transferred to TBS.

Then the sections were hybridized with the EBER PNA probe for one hour at 55°C. The simples were washed two times in 40% formamid in 5xSSC at 55°C for 15 min followed by washing in 5xSSC for 15 min at 55°C and two times in 2xSSC for 15 min for each at RT. Slides were treated for 1h with blocking solution (Maleic acid buffer 10% w/v) at RT. To enhance fluorescence and photostability the Alexa fluor 488 signal amplification kit (molecular probes) was used; the slides were incubated with Alexa fluor488 rabbit anti-FITC to bind FITC labeled probes and then with Alexa fluor 488 goat anti rabbit IgG for further enhancement. After washing steps, the slides were mounted with Dako fluorescence mounting medium (Dako USA). The samples were examined under an LSM 510 confocal microscope (Carl Zeiss, Germany).

Immunofluorescence

The expression of latent membrane protein 1 was examined by immunofluorescence in all gastric carcinoma cases having detectable EBV by FISH. The sections were subjected to heat antigen retrieval in citrate buffer (pH: 6) for 10 min as described previously [22],followed by washing in TBSx3 and then incubated with primary antibody anti LMP-1 (S12) 100 µl diluted 1:500 in PBS for 1hour. The samples were washed in TBSx3 times followed by incubation with the secondary antibody goat anti-mouse Alexa fluor 488 (1:1000) (Invitrogene USA) for 1h in the dark. After washing steps, stained sections were evaluated by confocal microscope. The phenotype of cells expressed LMP-1 was determined by two-color immunofluorescence using anti LMP-1 (S12) and rabbit polyclonal anti-cytokeratin (CK7, ab90083 from abcam) . The secondary antibodies used are goat anti-mouse Alexa fluor 488 for detection LMP-1 and goat anti-rabbit Alexa fluor 568 conjugated TRITC for detecting cytokeratine 7. Under confocal microscope, LMP-1 appears in green and cytokeratine 7 in red.

Immunohistochemical analysis

In order to confirm the results of immunofluorescence assays, immunohistochemical stains for LMP-1 were performed on all GC EBV positive samples. Known cases of LMP-1 positive Hodgkin lymphoma tissue was used as positive control. Paraffin sections pretreated with citrate for heat antigen retrieval, were incubated with the primary antibodies CS1-4 anti LMP-1 (1:200) for 1hour as described previously [22]. For detection protocol, the super sensitive non Biotin HRP Detection Kit EnVision (Dako Denmark) was used. Bound antibody was detected by diaminobenzidine chromogene (DAB, EnVision Kit) and tissues were counter-stained with hematoxylin.

Double labeling immunofluorescence and fluorescence in situ hybridization

In order to confirm the sensitivity of our FISH and IF protocols, we performed and end point double staining of LMP-1 by immunofluorescence and EBERs by fluorescence in situ hybridization. This technique was down on 15 cases of gastric carcinoma as described previously [23]with slight modification. Briefly, paraffin tissue sections were treated following dewaxing in xyline with proteinase K for predigesting step, the sections were then subjected to heat antigen retrieval in citrate buffer (pH: 6). The samples were incubated with primary antibody anti LMP-1 (S12) and then incubated with TRITC conjugated secondary antibody. The slides are then subjected to fluorescence in hybridization procedure with EBER PNA probe as described previously. Under confocal microscope, LMP-1 appears in red and EBERs in green.

Statistical analysis

For statistical analysis, we used the SPSS software. Relation of EBV status with independent variables including sex, age, tumor location and histological types was assessed by Chi square ($\chi 2$) and *P* values ≤ 0.05 is considered statistically significant.

RESULTS

Prevalence of EBV positive cases and association with clinico-pathological characteristics

Out of 97 patients with confirmed gastric carcinoma, 22 cases (22.7%) were proven to be positive for EBV by means of EBERs RNA fluorescence in situ hybridization. Excluding LELC subtype, the frequency of EBV associated gastric adenocarcinoma was (18.5%). FISH and HE staining in each adjacent pair of histological sections showed that EBERs were present within the malignant epithelial cells(Fig.1). EBERs were also observed in 2 cases of precancerous lesions showing dysplasia tissue, but in none of non-neoplastic cases. EBERs negative cancer tissues occasionally harbored scattered EBER-positive lymphocytes. Positive signals were bright green appeared as several small spots in cell nucleus of GC and in a limited number of infiltrating lymphocytes.

The characteristics clinico-pathologic of EBV-positive GC cases and EBV-negative GC cases were summarized in (Table 1).We have not found a significant association between EBV expression and gender. Indeed, among patients developing EBVaGC, the frequency of male was higher that the frequency of female, but the difference was not statistically significant(p=0.14).However, patients with EBVaGC were aged between 45 to 60 years old and this association was statically significant (p=0.029).With regard to anatomic site of the tumor, the positive rate of EBVaGC in antrum tumors (27%) was higher than that in fundus or body tumors (15.3%, 21%) but the difference was not significant (p=0.28). Analysis of histological features based on Lauren's classification, showed that there are not a significant difference between diffuse type EBV-positive cases and intestinal type(28%vs15%, p=0.13), but there was a significant difference between EBVaGC poorly differentiated grade and EBVaGC well and moderately grade (p=0.045).

Table1: Characteristics of EBV-positive GC cases and EBV-negative GC cases.

Clinical characteristics	Total (100%)	EBV expression		*p*-value
		Positive N (%)	Negative N (%)	
Gender				
male	67	18 (26.8)	49 (73.2)	*P*=0.14
female	30	4 (13.3)	26 (86.6)	
Age				
<45	29	4 (13.8)	25 (86.2)	
45-60	27	11 (40.8)	16 (59.2)	***P*=0.029**
>60	41	7 (17.0)	34 (82.9)	
Location				
fundus	26	4 (15.3)	22 (84.6)	
body	19	4 (21.0)	15 (79)	*P*=0.28
antrum	52	14 (27)	38 (73)	
Lauren type				
intestinal	40	6 (15)	34 (85)	*P*=0.13
diffuse	57	16 (28)	41 (72)	
differentiation Degree				
well	21	2 (9.5)	19 (90.4)	
moderate	19	3 (15.8)	16 (84.2)	***P*=0.045**
poor	57	17 (30)	40 (70)	

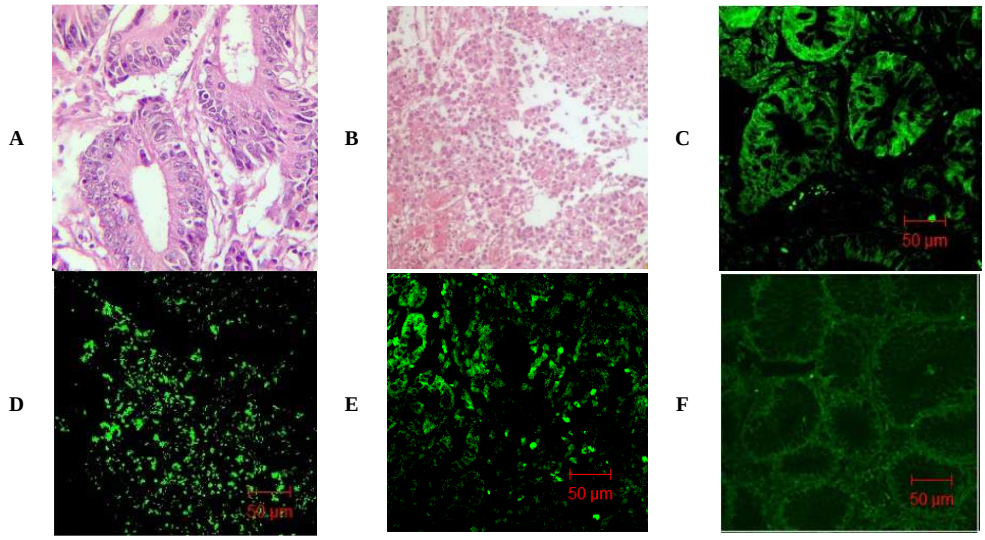


Figure 1. EBERs in situ hybridization in gastric carcinoma. A and B: Hematoxylin Eosin staining in adjacent sections for respectively C and D (original magnification x400). C: Positive signals for EBERs in well differentiated adenocarcinoma. D: Positive signals for EBERs in poorly differentiated adenocarcinoma. E: Positive signals for EBERs in moderately to poorly differentiated adenocarcinoma. F: Negative signal in well differentiated adenocarcinoma.

Of the intestinal types, EBV involvement was greater among moderately differentiated tubular adenocarcinoma as compared to well differentiated tubular adenocarcinoma (20% vs11.7%),(Table 2). In addition, of the diffuse type, solid poorly differentiated adenocarcinoma and signet-ring cell carcinoma have the highest proportions of EBV positive cases (34.8% and 20%) except lymphoepithelioma-like carcinoma cases which are known to be strongly associated with EBV (66.6%).In summary, EBVaGC in Algerian patients was associated with age at diagnosis and have preference of poor differentiation tumors.

Detection of latent membrane protein LMP-1

Two methods were used to evaluate the expression of LMP-1 in all 22 GC cases EBV positive. Immunofluorescence using S12 anti-LMP-1 revealed that LMP-1 was present in 8/22 (36.4%). IF staining showed the absent of LMP-1 expression in non-neoplastic cells and presence of expression in almost all carcinoma cells (Fig. 2 (a,b)). Two-color immunofluorescence of LMP-1 and cytokeratin defined the phenotype of LMP-1 expressing cells as epithelial cells (Fig. 2(c)).We performed immunohistochemical stains using CS1-4 anti-LMP-1 to confirm the presence of LMP-1 expression in EBVaGC samples. A high concordance was found between (IF) and (IH) staining with a similar trend: all 8 (100%) LMP-1 positive cases determined by IF were also confirmed to be positive by IH. All 14 negative cases by IF were also negative by IH.

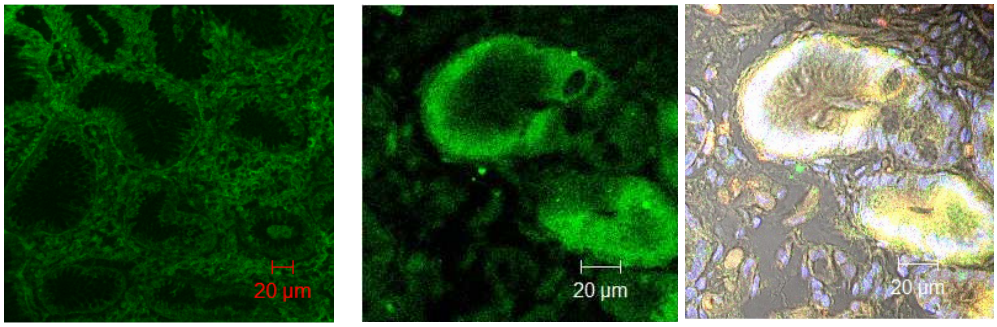
Immunohistochemical staining gives more morphological detail in the tissue compared to immunofluorescence, when present; LMP-1 was membranous/cytoplasmic, restricted to the malignant cells and absent in adjacent normal tissues as shown in (Fig. 3). Positive control using known LMP-1 positive Hodgkin lymphoma tissue, revealed a distinctive cytoplasmic and membranous stain.

Table 2. EBV associated gastric carcinoma by sex and histological type
*** Papillary adenocarcinoma. † Well differentiated tubular adenocarcinoma. ‡ Moderately differentiated tubular adenocarcinoma. § Solid type poorly differentiated adenocarcinoma. || Non-solid type poorly differentiated adenocarcinoma. # Signet ring cell carcinoma and special type of poorly differentiated carcinoma lymphoepithelioma-like carcinoma(LELC).**

Histological classification	Total		Male		Female	
	N	(%)	N	(%)	N	(%)
Intestinal	**6/40**	**(15)**	**5/30(16.6)**		**1/10**	**(10)**
pap*	0/3	(0)	0/3	(0)	0/0	(0)
tub1†	2/17	(11.7)	2/12	(16.6)	0/5	(0)
tub2‡	4/20	(20)	3/15	(20)	1/5	(20)
Diffuse	**16/57**	**(28)**	**13/37**	**(35.1)**	**3/20**	**(15)**
por1§	8/23	(34.8)	6/16	(37.5)	2/7	(28.5)
por2‖	2/18	(11.1)	2/13	(15.4)	0/5	(0)
sig#	2/10	(20)	2/5	(40)	0/5	(0)
LELC	4/6	(66)	3/3	(100)	1/3	(33)

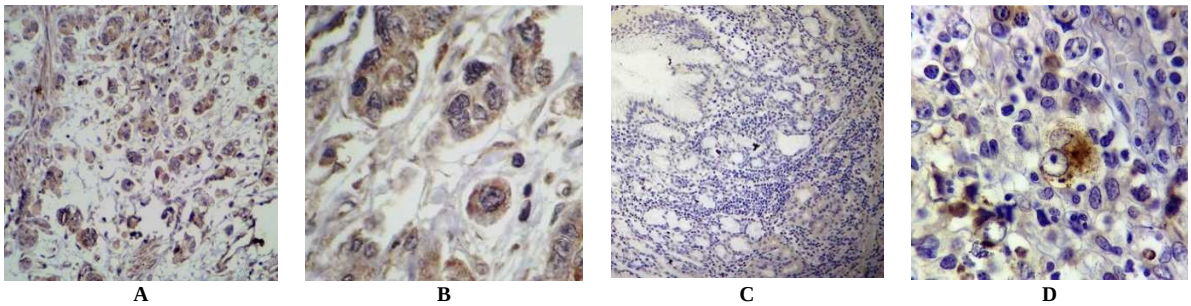
Co-localization of EBERs and LMP-1

Combined immunofluorescence for LMP-1 and fluorescence in situ hybridization for EBERs was carried out in 15 cases of gastric carcinoma. In five cases, double labeling was positive for both EBER and LMP-1. Ten cases were negative for EBER and negative for LMP-1. Double staining experiments and confocal microscopy revealed that LMP-1 expression was largely identical to the EBER positive signal. Overlaying the images showed co-localization of the LMP-1 and the EBER specific signals (Fig.4).



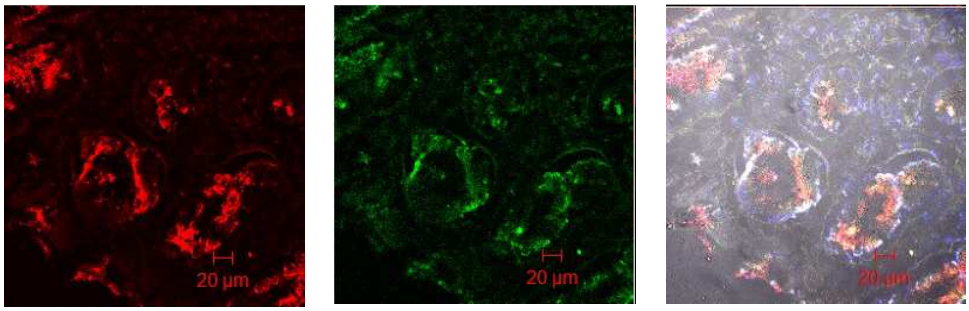
A **B** **C**

Figure 2: Immunofluorescence for latent membrane protein LMP-1. A: Negative signal in non-neoplastic gastric tissue. B: Positive signal found in well differentiated gastric carcinoma. C: Two-color immunofluorescence for simultaneous detection of LMP-1 (green) and cytokeratin (red) in well differentiated gastric carcinoma shows similar patterns of labeling (yellow).



A **B** **C** **D**

Figure 3:Immunohistochemical stain for LMP-1. A: LMP-1 expression in poorly differentiated adenocarcinoma EBV positive. B: Positive signal for LMP-1 is restricted to cytoplasmic and membrane of tumor epithelial cell (brown signal, full arrow). C: Negative signal observed in non-neoplastic gastric epithelium. D: Positive control shows LMP-1 expression in reed Sternberg cell of Hodgkin lymphoma. Original magnification (A, C x 400) and (B, D x 1000).



A **B** **C**

Figure 4:Dual staining immunofluorescence for LMP-1 and fluorescent in situ hybridization for EBERs shows. A: LMP-1 expression (red) and B: EBERs positive signal (green) in gastric carcinoma. C: Confocal analysis shows similar patterns of labeling and overlaying the images reveals colocalisation of the signals, yellow tissue section.

DISCUSION

This study is the first to determine the frequency of EBVaGC in cases from Algeria; we found a 22.7%prevalence of EBV related gastric carcinoma in Algeria. Among them, there were LEL carcinomas cases, which are strongly associated with EBV. Excluding this type of GC, the prevalence of EBV associated gastric adenocarcinoma was 18.5%; this frequency is among the highest frequency worldwide.

Actually it is well known that there are some regional differences in incidence of EBVaGC worldwide, Report from Asian countries example Japan and China, revealed a frequency comprises between 5.6 to 13.5%([24]; [25] ;[26]) ,it can reach up to 18% as shown in Germany([24]; [27]).In North Africa, only one paper published from Tunisia, which showed that the frequency of EBV related GC was 14.81%([28]).It seems that economic conditions are not an important variable for EBVaGC because low and high frequencies were found in both developed and developing countries ([29];[8]).We suggest that environmental and lifestyle factors, host genetic and strain variation of EBV are likely to have a role in this high prevalence of EBVaGC in Algeria. Further investigations are necessary in order to determine the strain of EBV in gastric carcinoma from Algerians patients and study the environmental factors in this area.

In this study, we found specific nuclear EBER1-2 transcripts in GC cells and in dysplastic gastric epithelium. Scattered EBER positive lymphocytes were found in some EBER-negative GC tissues which in shown also in previous report[30].In addition, we did not detected EBV in non-neoplastic gastric tissues. Our finding of EBV infection in precancerous lesion is in agreement with the result of Shibata and Weiss[4], and others authors([31];[32]).This finding supports the theory that EBV infection occurred before transformation and the mechanism of the entry of the virus into gastric epithelial cells are relate to previous damages and cooperation with some unknown promoter factors.

EBER in situ hybridization has become today the gold standard method for the detection of latent EBV infection in clinical formalin fixed paraffin embedded(FFPE) sections[33].In this study, Fluorescence in situ hybridization has used to detect EBER in GC using a commercially available EBER PNA probe. The probe is currently used to detect EBV infection in FFPE tissue specimens.[34]have used this probe in a FISH assay.[35]used EBER PNA probes in FISH assay and succeeded in simultaneously staining for both EBER and surface antigens. Recently, a study reported by[36]showed the efficiency and feasibility of FISH in the detection of EBER in FFPE tissue section of NPC. Subsequently, [37]tested FISH to detect EBV in GC biopsy specimens, they showed that this technique give a high sensitivity results even more slightly higher than that of chromogene in situ hybridization. Despite FISH showed less morphological detail in the tissue such as conventional ISH, but it can still localized EBER signal in gastric carcinoma cell.

About the clinico-pathological features of EBVaGC, we have observed an association with age of patients. Indeed, EBVaGC was more frequently ringed between 45 and 60 years. Previous study reported the association of EBVaGC with age. In fact,[38]showed that EBVaGC was highly associated with elder patients, while Herrera-Goepfert *et al.*, 2005.[32]reported that EBVaGC are common in younger age in Mexican population. However, other studies reported by [39]showed no association with age of patients. The second statistical association found in our study

was the tendency of EBVaGC with poorly differentiation tumors. These results are in agreement with those of several previous studies ([8]; [6]; [28]).

In addition, we don't observe a significant association between EBV expression and gender, tumor location and histology type. Ours results are similar to those observed in Tunisia[28].However, several studies have reported that patient with EBVaGC were predominately male and the tumor arise more frequently in the proximal stomach ([4]; [9];[10]).Furthermore, many studies reported that EBV expression exhibited significant association with diffuse type of GC ([10];[8];[6]).The difference between ours results and other reports can be attributed to the influence of the characteristics of the cohort, genetic background, environmental factor and life style on the development of EBVaGC.

LMP-1 is a 62-kd integral protein expressed in the cytoplasm and cell membrane. It plays a major role in cancer development by the ability to activate a wide range of signaling pathways such as NFκB and EGFR[15]. Recent studies confirm that LMP-1 is involved in the pathogenesis of some EBV related epithelial carcinoma as lake nasopharyngeal carcinoma[40].Other studies showed the presence of LMP-1 in cervical carcinoma from Algerian woman[41].Previously, it was found that LMP-1 can be secreted in the serum of NPC patients[42].

In our study, we have applied two different techniques using two different antibodies against LMP-1 in gastric carcinoma EBV positive, a high concordance was found between immunofluorescence using S12 antibody and immunohistochemistry using CS1-4 antibody. LMP-1 was observed in 8 of 22 EBVaGC, the expression was cytoplasmic and membranous in malign cells, and non expression was found in non-neoplastic gastric cells. In addition, we have applied double immunofluorescence staining of LMP-1 and cytokeratin; this technique defined the phenotype of LMP-1 expressing cells as epithelial cells.

The presence of LMP-1 in the EBVaGC was controversial. While some author's report that LMP-1 was present in their study.[43]demonstrated LMP-1 in 2 of 6 cases;[44],have found detectable amounts of LMP-1 expression in 3 of 12 cases of EBV positive GC. However, some studies showed absence of LMP-1 expression in GC ([9];[25];[45]).The lack of LMP-1 does not argue in a possible role for this viral protein in the pathogenesis of the tumor, but rather indicates that a continued LMP-1 expression may not be necessary once the malignant phenotype is established.

Using double staining FISH and IF, we are the first to detect simultaneously both EBER and LMP-1 in GC tissue. The technique was carried on 15 cases; five cases were simultaneously EBER positive and LMP-1 positive, ten cases were both EBER negative and LMP-1 negative. Double staining is more effective than either assay alone for identifying EBV related disease. The colocalisation of EBER and LMP-1 confirm the etiologic role of EBV in the pathogenesis of gastric carcinoma.

CONCLUSION

In conclusion, our study is the first to describe the frequency of EBVaGC in Algeria, highlighting a high prevalence. Our finding of the presence of EBV in pre-neoplastic tissue supports the hypothesis that EBV infection precedes transformation. Furthermore, LMP-1 expression indicate not only that EBV is present in latently infected cells which is similar to the type of EBV latency of nasopharyngeal carcinoma, but it supports the concept of an etiologic role of EBV in the pathogenesis. Further investigations are necessary in order to obtain information regarding the biological behavior of EBVaGC in Algerians patients.

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