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Detection of Epstein Barr Virus DNA in Gastric Adenocarcinoma in Brazzaville

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ABSTRACT

Introduction: The Epstein Barr virus (EBV) is associated with a variety of human malignancies including gastric cancer (GC).
Several studies have shown an association between Epstein-Barr virus (EBV) infection and the occurrence of many cancers in humans, including certain gastric carcinomas (GC). Indeed, recent studies have reported that 10% of GCs are associated with EBV. There is no study on the association of EBV with CG in Brazzaville.
The aim of the study was to detect EBV DNA in gastric adenocarcinoma in Brazzaville.
Materials and Methods: Samples of carcinomatous gastric tissues were analyzed by qPCR to detect EBV DNA. The samples were collected retrospectively between January 2008 and December 2018.
Results: Fifty two samples were analyzed. The PCR results showed that the detection of EBV DNA on our sample was 3.8%.
Conclusion: The results obtained from this preliminary study confirm the presence of EBV DNA in gastric adenocarcinoma, which is consistent with data from the literature.

Keywords: DNA, EBV, gastric adenocarcinoma, qPCR

INTRODUCTION

Gastric cancer is mainly represented by adenocarcinoma, which is the most frequent histological type (Bouvier AM et al; 2004). The latter develops from the gastric epithelium and remains a global public health problem (Bouvier AM et al; 2004). This pathology has variable prevalence and a poor prognosis. Worldwide, more than one million patients were diagnosed in 2018 (F. Bray et al 2018) and more than 780,000 deaths and two thirds in low-income countries (Klingelhöfer D et al 2020). According to the 2018-2019 Brazzaville cancer registry, the incidence rate of gastric cancer is 3.6 per 100,000 inhabitants.

The Epstein Barr virus (EBV), the first cause of human tumors of viral origin, was discovered 50 years ago. This virus is

considered a class 1 oncogenic pathogen by the World Health Organization (WHO). The presence of EBV in gastric cancer (GC) was first detected by Burke et al. (1990) in a rare form called undifferentiated carcinoma with lymphoid stroma identical to NPC. Later it was shown that all other histological types could be associated with EBV. (Shibata and Weiss., 1992)

The frequency of EBV-infected gastric cancer varies from 2 to 20%, with an average of 10%. (Shinozaki-Ushiku et al. 2015)

The objective of the study is to detect the presence of EBV DNA in gastric adenocarcinoma in Brazzaville.

MATERIAL AND METHOD

We conducted a descriptive study to collect retrospective data from tissue samples from gastric adenocarcinomas embedded in paraffin in the period from January 2008 to December 2018 at the Laboratory of Anatomy Pathology (LAP) of the University Hospital Center of Brazzaville (CHU-B). The inclusion criteria were all epithelial cancers diagnosed meeting the international recommendations of the American Society of Clinical Oncology (ASCO) and the American College of Pathologists (APC). All non-epithelial gastric cancers, poorly fixed, overfixed or insufficient samples were excluded.

The detection of EBV DNA was made by the molecular biology technique at the private laboratory Madeleine Gombes, according to the following method:

- **DNA extraction**

DNA extraction was done manually according to the procedure below:

Five micron sections were made using the Leica microtome from the paraffin blocks and put into the eppendorfs tubes. Deparaffinization was performed by adding 1 ml of xylene to each eppendorfs tube representing a sample. After vortexing for thirty seconds, the tubes were incubated at room temperature for fifteen minutes and then centrifuged at six thousand rpm at fifteen degrees Celsius for five minutes. After removal of the supernatant, one milliliter of seventy percent ethanol was added, vortexed, incubated for fifteen minutes at room temperature and then centrifuged for five minutes at six thousand rpm at fifteen degrees Celsius. The operation was repeated twice. Finally, the pellet was dried at room temperature overnight. The deparaffinized and dried pellet was washed with PBS (phosphate buffered saline) twice until residual ethanol was not visible. The samples were resuspended in one hundred and eighty microliters of tissue lysis buffer with the addition of twenty microliters of proteinase K, then vortexed. Incubation occurred at fifty six degrees Celsius for one hour following the manufacturer's instructions. The samples were then cooled to temperature ambient. Then, we performed two to three washes by adding five microliters of washing solution followed by centrifugation at fourteen thousand revolutions for two minutes. After vacuum centrifugation, fifty to one hundred microliter of elution solution was added to each collection tube containing a filter followed by an initial gentle centrifugation at two thousand rpm for one minute, then a high-speed centrifugation eight thousand revolutions per minute for two minutes. The collected DNA was immediately stored at -20°C for later use. DNA extraction was performed using the DNeasy® Blood & Tissue kit (QIAGEN) as described by the manufacturer. A Qubit® 3.0 spectrophotometer was used to read the concentrations.

- **Quantification by real-time PCR**

Quantification of Epstein Barr Virus DNA was determined using a "Diagenode® EBV) kit". The kit includes hydrolysis probes for the Epstein-Barr virus Diagenode, primers and hydrolysis probe for the Diagenode inhibition control and a Diagenode plasmid inhibition control. Real-time PCR amplification was performed in a

25 µl volume containing 12.5 µl qPCR Mix, 2.5 µl primers, 5 µl molecular biology water, and 5 µl each sample and controls. Amplification and detection were performed by a real-time PCR machine (Magnetic Induction Cycler: Mic). Thermal cycles used for detection of EBV DNA were amplified for initial denaturation at 95°C for 10 minutes, followed by 45 cycles of 95°C for 15s and hybridization at 60° for 60s.

Table 1: Magnetic Induction Cycler Program

Level	Description
1	2 minutes at 50°C (1 cycle)
2	10 minutes at 95°C (1 cycle)
3	Cycle program (45 cycles) Stage 1 : 15 seconds at 95°C Stage 2 : 60 seconds at 60°C

Statistical analyzes

We used Microsoft Excel 2010 software to compile the database. Quantitative variables were expressed as mean ± standard deviation and qualitative variables were expressed as numbers and percentages

RESULTS

Fifty-two cases were eligible for EBV DNA detection. The results were as follows: the age of our patients was between 23 and 86 years old. A male predominance is observed with 34 men and 18 women, i.e. a sex ratio of 1.9. Detection of Epstein Barr Virus DNA was made by real-time quantitative polymerase chain reaction in 3.8% of cases. Both cases were male and over 50 years old and the fundus was the primary location.

Table 2: Distribution of EBV DNA in gastric ADK according to age

	Présence de l'EBV	Absence de l'EBV
< 50	0	21
≥ 50	2	29
Total	2	50

Presence of EBV DNA in gastric ADK and sex

Table 3: Distribution of EBV DNA in gastric ADK according to sex

	Presence of EBV	Absence of EBV
Male	2	32
Féminine	0	18
Total	2	50

Presence of EBV DNA in gastric ADK and histological type

All cases of gastric adenocarcinoma with the presence of EBV DNA were of the diffuse type.

Presence of EBV DNA in gastric ADK and localization

All cases of gastric adenocarcinoma with the presence of EBV DNA had the fundus as location.

Presence of EBV in gastric ADK and histoprognostic grade

All cases of gastric adenocarcinoma with the presence of EBV DNA were poorly differentiated.

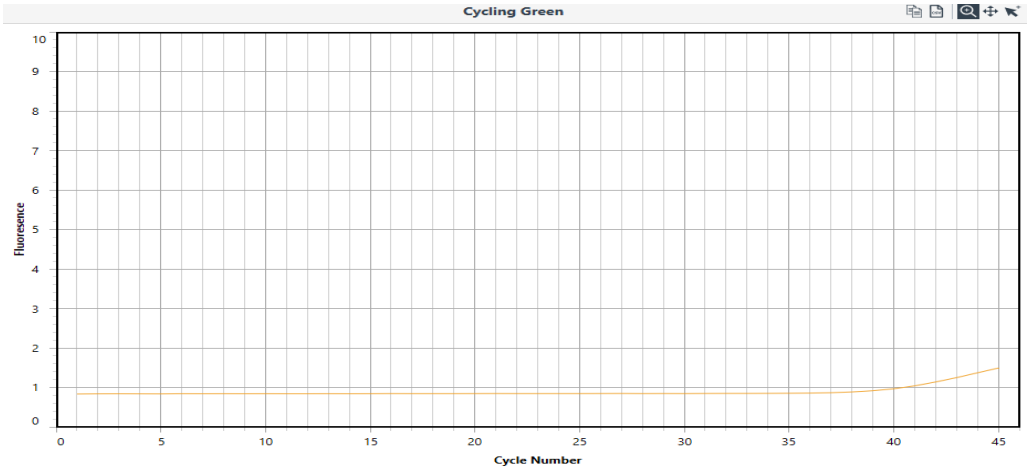

Figure 1: EBV DNA positive case in gastric cancer (qMIC result)

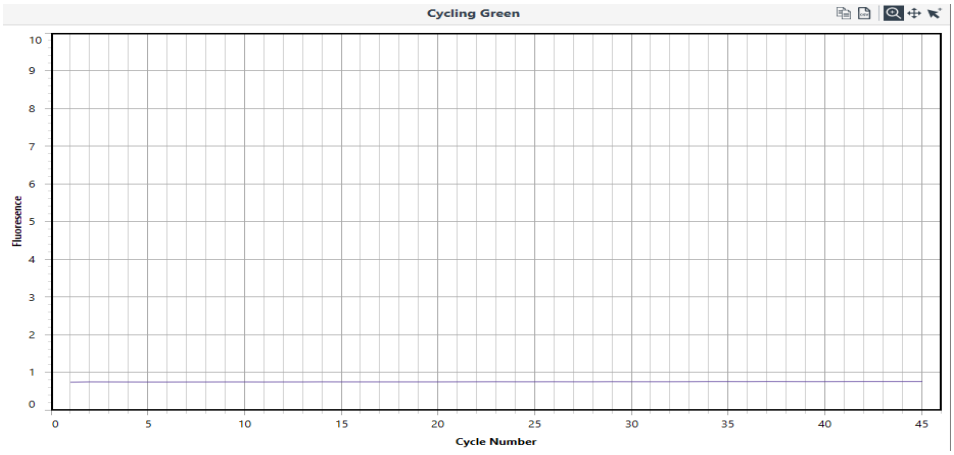

Figure 2: EBV DNA negative case in gastric cancer (qMIC result)

DISCUSSION

The presence of EBV DNA in gastric adenocarcinoma in our study was 3.8%, which corroborates with the hypothesis of our study which was to verify the presence of Epstein Barr virus in adenocarcinoma gastric.

EBV-associated gastric adenocarcinoma occurs worldwide, with significant geographic variation (Takada, 2000). According to meta-analyses, the frequency of EBV infections in gastric cancer ranges from 2 to 20 %, with a global average of around 10% (Xu and *al* 2016; Wang and *al* 2016(a)).

Our results are similar to those found by Camago and al in 2011 in China who found a frequency of 4.3%.

Takada and *al* in 2000 in Japan found a 6.9% rate of EBV associated with gastric adenocarcinoma.

However, our results are very discordant with those obtained by Shibata and al in 1993, Gulley and *al* in 1996 in the United States who found a high frequency of EBV linked to gastric adenocarcinoma of 16%.

In Germany Ott and *al* in 1994 and Ryan et al found a very high frequency of 18%.

In view of the above, our results are related to the hypothesis of our work

In our study, all of our cases were over 50 years old, which is what emerges in the study by Paul H and *al* (Paul H and *al*, 1995).

Roberto Herrera-Goepfert et al in Mexico had found results similar to ours where the cases of gastric tumors associated with EBV were over 50 years old. (Roberto Herrera-Goepfert and *al*, 2005)

Detection of EBV DNA was present only in male cases. This is evident in the study by Shibata and al where EBV was most often detected in gastric tumors in men compared to women. (D. Shibata and *al*, 1992)

In relation to the presence of EBV in the gastric ADK and the histological type, all of our gastric tumors associated with EBV were of the diffuse type; this is in line with the studies of E Yamamoto and al, and MC Camargo (E Yamamoto and *al*, 2011; MC Camargo and *al* 2011)

CONCLUSION

EBV has been closely linked to a wide range of tumors of both lymphoid and epithelial origin. Advances in the molecular analysis of EBV have revealed fundamental mechanisms in the relevance of the oncogenic process. This virus provides a paradigm for the exploitation of knowledge at the molecular level, in the diagnosis, treatment and prevention of cancer. This work allowed us to demonstrate for the first time the presence of EBV DNA in gastric adenocarcinoma in Brazzaville.

In order to continue this work, it would be interesting in a future investigation to carry out a study on a large number of gastric tumors from different

regions in order to assess the real prevalence of gastric cancers associated with the Epstein Barr Virus. On the other hand, we believe that additional work is necessary to study:

- cases using the In Situ Hybridization technique
- the profile of the expression of all the latent or lytic proteins of EBV, which could make it possible to better understand the molecular mechanisms by which this virus contributes to the carcinogenesis of gastric cancer.

All this will contribute to improving the management of gastric adenocarcinoma in Congo Brazzaville

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Ethical Approval: Approved

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