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# The Frequency of Human Papillomaviruses and Epstein-Barr Virus in Colorectal Cancer Samples in Algeria

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## ABSTRACT

**Background:** Environmental factors may play a role in colon cancer. In this view, several studies investigated tumor samples for the presence of various viral DNA with conflicting results. The purpose of this study is to investigate the prevalence of Human papillomaviruses (HPVs) and Epstein-Barr virus (EBV) in patients with colorectal carcinomas.

**Materials and Methods:** In this study, we collected 74 tumorous paraffin-embedded tissues (Mean±SD age: 66.3±14.98) from the Pathology Department of a hospital in Ain Temouchent and laboratories of pathological anatomy in western Algeria. DNA from each tissue was extracted and the presence of HPV and EBV was investigated using PCR.

**Results:** None of our samples were HPV or EBV positive, and we failed to find an obvious correlation between EBV and HPV infections and this type of cancer.

**Conclusion:** The results suggested that EBV and HPV infection is not common in patients with colorectal cancer in our population. However, the findings merit more investigations on a large number of cases.

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## Introduction

Cancer is a major public health problem worldwide. It is one of the leading causes of death in several regions depending upon disparities among different people [1].

Colorectal cancer is the 3rd most common cancer, estimated at 1.9 million new cases in 2020 which accounts for approximately 10% of all cancer cases according to the World Health Organization GLOBOCAN database [2]. Colorectal cancer (CRC) is considered the 4th primary cause of cancer-related deaths in males and the 3rd cause of cancer-related mortality among females [2].

CRC is a tumor that occurs in the colon, the rectosigmoid junction, the rectum, and the anus. In the majority of cases, CRC develops on the pedunculated, much less in the non-pedunculated adenocarcinomas. In the metaplastic processes, non-invasive cancer develops only in the mucosa, and afterward invasive cancer beyond the lamina propria of the mucosa [3].

Neoplastic progression can be influenced by different genetic and environmental factors, that is the objective of a meta-analysis performed by Johnson et al. Showing that the risk of developing CRC decreases due to various factors as follows: inflammatory bowel disease (RR=2.93 [1.79-4.81]); hereditary familial history (RR=1.80 [1.61-2.02]); body mass index (RR=1.10 [1.08-1.12]); smoking cigarettes (RR=1.06, [1.03-1.08]); and physical activity (RR=0.88 [0.86-0.91]) [4].

However, around 20% of all cancers are acknowledged to be caused by infectious agents including bacteria and viruses. A relatively small number of viruses including human papillomavirus (HPV), hepatitis B virus, hepatitis C virus, and Epstein-Barr virus (EBV) can cause several types of cancer such as Merkel cell, cervical cancer, Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinomas [5].

Recently, several studies have suggested that viruses such as human papillomavirus (HPV), JC, BK, and EBV may be related to CRC [6]. HPVs are nonenveloped, double-stranded DNA viruses that infect the basal layer of epithelial cells [6]. HPVs are divided into two types: low-risk and high-risk HPVs [7]. Persistent infection with high-risk HPVs can result in the development of intraepithelial neoplasia (pre-cancerous lesions) that can progress to invasive cancer including cervical, anal, penile, esophageal, and vaginal cancers [8].

The HPV genome consists of a circular double-stranded DNA molecule of approximately 8000 base pairs, with eight open reading frames, all of which are transcribed from the same DNA strand and in the same orientation. The genome is organized into three main regions [9] including the E region encoding six genes involved in multiple functions, including viral replication and cell transformation (E1, E2, E4, E5, E6, and E7), the L region encoding L1 and L2 capsid proteins that self-assemble into the virion [10] and long control region which contains activators and promoters of the sequences [10].

The integration of viral DNA into the cellular genome results in partial or total loss of the E2 gene, resulting in increased expression of the HPV E6 and E7 oncogenes. Overexpression of E6 and E7 oncoproteins leads to the degradation, respectively, of p53 and pRb, inducing HPV carcinogenesis. In addition, E6 causes the activation of telomerase, deregulating the pathways involved in cell proliferation, differentiation, immune recognition, and survival signaling. E7 increases genomic instability, resulting in the accumulation of chromosomal changes. This deregulation of the cell cycle, activation of telomerase, and induced genomic instability create a favorable environment for the neoplastic transformation of cells. It is known that these molecular processes lead to genomic instability and cell transformation, an indicator mechanism of HPV-induced colorectal carcinogenesis [11].

The EBV virion has a structure common to all herpesviruses. It is spherical, measuring between 120 and 200 nm in diameter with a 184-kbp long, double-stranded DNA genome that encodes more than 85 genes [12], including viral oncogenes such as six EBV-encoded nuclear antigens (EBNA1, -2, -3A, -3B,-3C, and -LP) and latent membrane proteins (LMP1, -2A, and -2B), as well as various noncoding RNAs (EBERs and miRNAs) [12].

The viral particle attaches specifically to the cell surface through a high-affinity interaction between the gp350/220 of the viral envelope and the CD21 molecule present on the plasma membrane of B lymphocytes. The attachment of the virus to CD21 induces the initial signals for cell activation and endocytosis. The viral envelope fuses with the cell membrane and this penetration require the interaction of the gp85-gp42 complex. EBV can use the following molecules as co-receptors human leukocyte antigen class II. The mechanism of infection for other cell types is different from that of B lymphocytes [13].

The EBV can replicate in two ways by infecting B cells (latent form) and by lytic production of the virion [14]. All EBV-associated cancers involve the virus's latent cycle. Four types of latent gene expression have been described [15].

The virion components are excessively amplified (up to 1000 times) in the nucleus, the lytic program blocks the cell cycle and cellular processes with the help of the transcription factors BZLF1 and BLIMP1, the latter being necessary for uncontrolled replication in epithelial cells, which may become neoplastic [14]. This study aimed to evaluate the presence of HPV and EBV in the CRC in the Algerian population.

## Materials and Methods

The research materials consisted of paraffin sections of retrospectively chosen colorectal cancer tissue taken from histopathological specimens from 74 patients who were referred to the Pathology Department of the hospital of Ain Temouchent and the laboratories of pathological anatomy of western Algeria. From the patient's medical records we obtained data on the age, sex, and type of tumor. The study population is described in Table 1. A total of 74 samples were included in this study, 44 female and 30 male. The Mean±SD age was 66.3±14.98 years and the patients' age ranged between 25 and 98 years. Five sections of 10-20 µm of the paraffin-embedded samples were prepared with a stained slide to determine the tumor area by a pathologist.

### DNA extraction

Genomic DNA was deparaffinized and extracted from paraffin-embedded tissue samples using Purigene Kit (Qiagen, Hilden, Germany) with modifications to optimize DNA yield. Briefly, 900 µL of xylene was added to samples to remove the paraffin, followed by 900 µL of 100% ethanol. Samples were then incubated at 55°C in a lysis solution and proteinase K overnight, an incubation step at 90° for one hour with a dose of proteinase k is added for complete lysis. After samples were completely lysed, a protein precipitation solution was added. The DNA pellet was washed with ethanol 70%, and the DNA sample was dissolved in 20 µL of DNA hydration solution and incubated at 65°C for 1 hour. To determine the purity of the sample wavelength of 280/260 was examined. The extracted DNA was stored at -20°C until use.

### PCR methods

Specific PCR was carried out based on HPV L1 consensus primers MY09/MY11 (MY09: 5'-CGTCCMARRGGAWACTGATC-3', and MY11: 5'-GCMCAGGGWCATAAYAATGG 3') [16].

The PCR amplification was performed in a 25 µL reaction volume containing 2.5 µL of PCR buffer, 0.6 µL of forward and reverse primers, 0.5 µL of dNTP, 2.5 U/µL of Taq, 19.25 µL of $H_2O$, and 1 µL of each genomic DNA sample. The PCR program was performed as follows: pre-denaturation at 95°C for 15 minutes, denaturation at 95°C for 30 seconds, annealing at 53°C for 45 seconds, extension at 72°C for 1 minutes, 40 cycles, and post-extension at 72°C for 5 minutes, 1 cycle. At the end of amplification, 1 µL of the PCR product was analyzed on 1.5% agarose gel. The resultant product was expected to be a 450-bp fragment. We chose to amplify the region of the EBV LMP1 gene with the following primers:

F:5' AATAGACAGCCCAGTTGAAA 3' R:5' GCAGTGCCATATCTGACGTG 3' [17],

Negative control samples containing water instead of DNA were always processed in a manner parallel to the patient's samples.

The PCR amplification was performed in a 25 µL reaction volume containing 2.5 µL of PCR buffer, 0.6 µL of forward and reverse primers, 0.5 µL of dNTP, 2.5 U of Taq, 19.25 µL of $H_2O$, and 1 µL of each genomic DNA sample. The PCR program was performed as

**Table 1.** Frequency distributions of selected variables in colorectal cancer cases (N=74)

| Variables | | No. (%) |
|---|---|---|
| Gender | Female | 44(59.5) |
| | Male | 30(40.5) |
| Age | >50 | 10(13.5) |
| | 51-60 | 21(28.4) |
| | 61-70 | 15(20.3) |
| | <70 | 28(37.8) |
| Site of tumor | Colon | 58(78.4) |
| | Rectal | 16(21.6) |

follows: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, extension at 72°C for 3 minutes, 35 cycles, and post-extension at 72°C for 5 minutes, 1 cycle. At the end of amplification, 1 µL of the PCR product was analyzed on 2% agarose gel. The resultant product was expected to be a 509-bp fragment.

### Statistical analysis

The data collected were cleaned and analyzed with SPSS version 25 software. $P<0.05$ was used as the significance criterion.

## Results

The DNA was efficiently extracted from the samples. All samples (74 colorectal adenocarcinomas) were negative for HPV DNA and EBV DNA.

## Discussion

To the best of our knowledge, this is the 1st study reporting the presence of HPV and EBV in human CRC in the Algerian population. CRC is a real public health problem, both in Algeria and throughout the world by its frequency and high mortality. More than a 3rd of new cases of CRC occur outside of industrialized countries. The evaluation of the epidemiological profile has shown that CRC in our region is the 3rd most incidence of cancer after breast and thyroid cancer among females (10.7/100,000 inhabitants) and after bladder and prostate cancer among males (14.3/100,000 inhabitants) [18].

A considerable number of studies are focused on recognizing novel genetic alterations included in CRC pathogenesis, the examinations have appeared that infectious diseases, specifically viral diseases, may also be considered as a primary basic reason involved in the formation of this cancer [19].

The data suggesting an impact of an HPV infection on the development of CRC appeared in 1990. The prevalence rates of HPV in CRC vary according to the available data, ranging from 0% [19-23] to 85% [20].

PCR is the most used technique for the detection of high-risk viral DNA in fresh or formalin-fixed and paraffin-embedded tumor tissue [21].

In our study, HPV DNA positivity was not found in any of our cases with adenocarcinoma and adenoma. Probably the low number of samples in this study caused this result which is in agreement with a study carried out in Turkey in 2011 [22]. Other studies carried out in Iran and Portugal respectively declare that HPV DNA was not identified in any of the normal, adenocarcinoma, or adenoma samples [23, 24]. According to the study of Taherian et al. [25], which is a meta-analysis of many research studies published between 1992-2020, detecting HPV in CRC in different regions of the world, the prevalence of the virus fluctuates from 0-83% with the mean value of 39.05%.

Our findings are in contrast with other studies in this field that have highlighted the presence of HPV in simple CRC. Studies using PCR from Saoudi Arabi, Iran, and Syria revealed the presence of HPV in 0.8% [26], 1% [27], and 37%, respectively [21]. In addition, a study in Poland declare that HPV DNA was detected in 20% of cases [28].

The highest reported HPV positivity in CRC was detected in the USA (51%) [29], Argentina (74%) [30], Brazil (83%) [31], Lebanon (64%) [32], and Turkey (83%) [33]. EBV is a human herpes virus that infects more than 90% of the world's population [34]. It has been described to be associated with several human cancer diseases such as nasopharyngeal and gastric carcinomas and various lymphomas, such as Burkitt's lymphoma, NK/T lymphomas, and some Hodgkin's lymphomas [35]. When it comes to CRC, several studies have established a causative link between EBV and colorectal carcinogenesis [36, 37].

Accordingly, our present study in the Algerian population failed to detect the presence of EBV in human CRC samples. In parallel with our result, Boguszakova et al. [38] also failed to detect EBV DNA in the biopsy specimens from adenocarcinoma/adenomatous colorectal tissues. Similarly, Cho et al. reported no sign of EBER gene expression in colorectal tumor specimens, from 274 Korean patients [39]. A study carried out in Sudan showed no relationship between EBV and CRC tissues [34]. An Italian study also declares that any case of CRC samples is EBV positive [40]. In Iran, reports demonstrated the absence of a significant correlation between EBV and CRC development [12, 37].

In concordance with data obtained in the present study, some other studies found a correlation between EBV and human CRC. A study in Iran declared that EBV DNA was detected in only 1 out of 70 (1.4%) adenocarcinoma colorectal tissues while the rest were negative [41]. Karpinski et al. reported that 19% of the CRC cases from Poland were EBV-positive [42]. In Iraq, 20% of the

samples were EBV-positive [43]. A Chinese cohort of colorectal carcinomas was reported to be EBV-positive in 30% of the samples [44].

Significant differences were observed in the detection rate of EBV in Syria and Iran with 36% [45] and 38% [46] of the cases, respectively. The highest reported EBV-positivity in CRC was detected in Chile (46%) .

## Conclusion

The results suggested that EBV and HPV infection is not common in patients with CRC in our population. The difference in the positivity rates, observed in the studies, can be explained by some factors, such as the different methods of HPV detection and the choice of the materials for analysis (fresh/paraffin). However, the findings merit more investigations on a large number of cases. A reliable and definite proof will provide new opportunities for the prevention, diagnosis, and therapy of colorectal cancer.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by the Ethics Committee of Abou Bekr Belkaid University (Code: 174.FM.UABB.24).

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### Authors' contribution

Contribution to all experimental work, data, and statistical analysis, and writing the manuscript: Bouchra Dahmani; Data analysis and investigation, Writing–original draft, and , and Writing–review: Lamia Boublenza, Nafissa Chabni, and Hafida Hassaine; Data collection: Dalale Behar; Investigation: Belkacem Belatbi and Amel Benfoula; Conceptualization and Supervision: Ikram Breik; determined the tumor area of colorectal samples: revised the manuscript; helped to edit the manuscript, and all authors read and approved the final manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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