

RESEARCH ARTICLE

Open Access



Serum EBV antibodies and LMP-1 in Polish patients with oropharyngeal and laryngeal cancer

Sylwia Fołtyn¹, Małgorzata Strycharz-Dudziak^{2*}, Bartłomiej Drop³, Anastazja Boguszewska¹ and Małgorzata Polz-Dacewicz¹

Abstract

Background: The association between Epstein-Barr virus (EBV) and the development of head and neck cancer was reported by many researchers. The aim of the present study was to detect EBV DNA and EBV antibodies in 110 Polish patients with oropharyngeal and laryngeal cancer compared to 40 healthy individuals.

Methods: Frozen tumor tissue fragments were tested using nested PCR assay for EBV DNA detection. Sera from all individuals were investigated using ELISA tests to detect the presence of VCA IgM and IgG, EBNA IgG, EA IgG.

Results: EBV DNA was detected in 52.7% of the patients (25% in controls). EBVCA were detected in 94.5%, EBNA in 96.4% and EA in 94.5% of patients. The significantly higher level of EA in the patients suggests EBV reactivation. The majority of patients (83%) were infected with wild-type EBV.

Conclusion: Our study showed that this variant seems to be associated with oropharyngeal and laryngeal cancer in the Polish population.

Keywords: EBV antibodies, LMP1, Oropharyngeal cancer, Laryngeal cancer

Background

The Epstein-Barr virus (EBV) is a ubiquitous gammaherpesvirus that infects more than 90% of the global adult human population [1–3]. For the past two decades, increasing interest has been focused on the EBV-associated cancers including Burkitt's lymphoma (BL), Hodgkin lymphoma (HL), nasopharyngeal carcinoma and gastric cancer [4–8].

The global burden of mortality from EBV-related cancers accounts for 1.8% of all cancer deaths in 2010 [9]. The trends indicate that both this burden and life-expectancy in the world population will increase. About 92% of all EBV-associated cancer deaths are caused by nasopharyngeal cancer and gastric cancer. After primary infection, EBV establishes latent infection in B lymphocytes with periodic reactivation and viral transmission in the oropharyngeal epithelium. Chronic viral infection is

an important risk factor, particularly for tongue cancer and oropharyngeal cancer [1, 7, 8, 10–14]. The association between the latent EBV infection and the development of head and neck cancer (HNC) was reported by several investigators [11, 12, 14, 15].

The present study analysed the serum level of EBV antibodies (VCA IgM and IgG, EBNA IgG, EA IgG) and determined LMP1 variant in patients with oropharyngeal and laryngeal cancers in the Polish population.

Methods

Patients

The present study comprised of a group of 110 patients with a diagnosed cancer of the pharynx or larynx who were hospitalized in Otolaryngology Division of the Hospital in Radom, Poland. The results were compared to the control group, involving 40 persons hospitalized at the Otolaryngology Ward due to diseases other than cancer. There were no statistically significant differences between the patients and the control group (age, sex, tobacco and alcohol consumption (Table 1)). The socio-

* Correspondence: malgorzata.strycharz-dudziak@umlub.pl

²Chair and Department of Conservative Dentistry with Endodontics, Medical University of Lublin, Lublin, Poland

Full list of author information is available at the end of the article

Table 1 Epidemiological characteristics of patients and control group

| | | Patients (n = 110) | | Controls (n = 40) | | p |
|--------------------|----------|-----------------------|--------|----------------------|------|--------|
| | | n | % | n | % | |
| | | Sex | female | 8 | 7.3 | |
| | male | 102 | 92.7 | 38 | 95.0 | |
| Age | <49 | 12 | 10.9 | 6 | 15.0 | >0.05 |
| | 50–59 | 46 | 41.8 | 16 | 40.0 | |
| | >60 | 52 | 47.3 | 18 | 45.0 | |
| Place of residence | urban | 72 | 65.5 | 28 | 70.0 | >0.05 |
| | rural | 38 | 34.5 | 12 | 30.0 | |
| Smoking | yes | 94 | 85.5 | 36 | 90.0 | >0.05 |
| | no | 16 | 14.5 | 4 | 10.0 | |
| Alcohol abuse | yes | 50 | 45.5 | 14 | 35.0 | >0.05 |
| | no | 60 | 54.5 | 13 | 65.0 | |
| EBV DNA | positive | 58 | 52.7 | 10 | 25.0 | <0.001 |
| | negative | 52 | 47.3 | 30 | 75.0 | |

demographic and clinicopathological characteristics of the study group are shown in Table 2 in relation to EBV DNA. The research material consisted of the sera and frozen tumor tissue fragments. This research was approved by the Ethics Committee and is in accordance with the GCP regulations (no. KE-0254/133/2013).

DNA extraction from fresh frozen tumour tissue

Fragments of the fresh frozen tumour tissue (20 mg), both from the patients with OSCC and from the control subjects (biopsies), were cut and homogenized in a manual homogenizer Omni TH/Omni International/Kenneseewa/Georgia/USA. DNA was extracted using a protocol as described in the DNeasy Tissue Kit Handbook (QiagenGmbH, Hilden, Germany). Purified DNA was quantified by spectrophotometry (Epoch Microplate Spectrophotometer, BioTek Instruments Inc., Winooski, Vermont, USA). The isolates were kept at -20°C until the test was conducted. To verify the quality of the obtained DNA (presence of inhibitors of Polymerase Chain Reaction), a β -globin assay was performed.

Detection of viruses

EBV DNA detection: All PCR reactions were carried out in the final volume of 25 μl using HotStartTaq DNA Polymerase (Qiagen, Germany). Concentrations of PCR reaction components were prepared as follows: 2.0 mM MgCl_2 , 0.2 mM dNTPs, 0.5 μM of each forward and reverse primers and 0.5 U of HotStartTaq polymerase. During each run the samples were tested together with one negative (nuclease-free water) and positive control (EBV-positive cell line, Namalwa, ATCC-CRL-1432).

Table 2 Epidemiological and clinical characteristics of patients in relation to EBV DNA

| | | EBV DNA+ | | EBV DNA- | | p |
|----------------------|----------|----------|--------|----------|-------|--------|
| | | n | % | n | % | |
| | | Sex | female | 0 | 0 | |
| | male | 58 | 56.9 | 44 | 43.1 | |
| Age | <49 | 8 | 50.0 | 4 | 50.0 | 0.8969 |
| | 50–59 | 22 | 47.8 | 24 | 52.2 | |
| | >60 | 28 | 50.0 | 24 | 50.0 | |
| Place of residence | urban | 40 | 55.6 | 32 | 44.4 | 0.5630 |
| | rural | 18 | 47.4 | 20 | 52.6 | |
| Smoking | yes | 50 | 53.2 | 44 | 46.8 | 0.8672 |
| | no | 8 | 50.0 | 8 | 50.0 | |
| Alcohol abuse | yes | 28 | 56.0 | 22 | 44.0 | 0.6571 |
| | no | 30 | 50.0 | 30 | 50.0 | |
| Histological grading | G1 | 22 | 61.1 | 14 | 38.9 | 0.6852 |
| | G2 | 34 | 48.57 | 36 | 51.43 | |
| | G3 | 2 | 50.0 | 2 | 50.0 | |
| T stage | T1 | 6 | 42.9 | 8 | 57.1 | 0.1826 |
| | T2 | 28 | 58.3 | 20 | 41.7 | |
| | T3 | 16 | 72.7 | 6 | 27.3 | |
| | T4 | 8 | 30.8 | 18 | 69.2 | |
| N stage | N0 | 30 | 51.7 | 28 | 48.3 | 0.9768 |
| | N1 | 10 | 50.0 | 10 | 50.0 | |
| | N2 | 14 | 58.3 | 10 | 41.7 | |
| | N3 | 4 | 50.0 | 4 | 50.0 | |
| M stage | M0 | 58 | 52.7 | 52 | 47.3 | |
| | M1 | 0 | 0 | 0 | 0 | |
| Location of cancer | pharynx | 23 | 50.0 | 23 | 50.0 | 0.6452 |
| | larynx | 35 | 54.7 | 29 | 45.3 | |
| EBV type | wild | 48 | 82.8 | - | - | - |
| | del-LMP1 | 10 | 17.2 | - | - | |

^{*}statistically significant

Amplification of EBNA-2 gene

The nested PCR was carried out for amplification of EBNA-2. The sequence of primers used for PCR was as follows: outer pair 5' – TTT CAC CAA TAC ATG ACC C – 3', 5' – TGG CAA AGT GCT GAG AGC AA – 3' and inner pair 5' – CAA TAC ATG AAC CRG AGT CC – 3', 5' – AAG TGC TGA GAG CAA GGC MC – 3'. 2 μl of extracted DNA was subjected to the PCR mixture with the concentration as described above. The first-round amplification consisted of the activation of polymerase 95°C for 15 min, 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and the final extension at 72°C for 5 min. The second-round amplification was performed with 1 μl of first round PCR product in 30 cycles with an annealing temperature at 60°C . The

amplicons 368 bp, 473 bp in length (depending on the EBV type EBV-1 and EBV-2, respectively) were separated on 2% agarose gel and purified using a Gel-Out kit (A&A Biotechnology, Poland) for further analysis. Purified PCR products were sent to Genomed Warsaw company for sequencing.

Genotyping of LMP1

PCR screening for the EBV LMP1 subtype based on exon 3, defined as wild-type (wtLMP1) or del-LMP1, was done using specific primers: forward 5'-AGC GAC TCT GCT GGA AAT GAT- 3'; revers 5'-TGA TTA GCT AAG GCA TTC CCA- 3'. Concentrations of PCR reaction components were prepared as follows: 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each forward and revers primers and 0.5 U Hot Start DNA polymerase and 5 μl of extracted DNA. The reaction mixture (25 μl) was incubated at 95 °C for 15 min., followed by 45 cycles at 94 °C for 1 min., 57 °C for 1 min., 72 °C for 1 min., a final extension at 72 °C for 10 mins. The PCR products were analyzed by gel electrophoresis in a 3% agarose gel and visualized under UV light.

Serological tests

To detect antibody levels serological tests were used with ELISA method. Designed antibodies: anti-VCA IgM (NovaLisa Epstein-Barr Virus VCA IgM/Nova Tec Immunodiagnostica GmbH/Germany/catalog number: EBVM0150), anti-VCA IgG (NovaLisa Epstein-Barr Virus VCA IgG/Nova Tec Immunodiagnostica GmbH/Germany/catalog number: EBVG0150), and anti-EBNA IgG (NovaLisa Epstein-Barr Virus EBNA IgG/Nova Tec Immunodiagnostica GmbH/Germany/catalog number: EBVG0580), antibodies anti-EA IgG (ELISA-VIDITEST anti-EA (D) EBV IgG/Vidia/Czech Republic/catalog number: ODZ-006). All tests were performed according to the manufacturer's instructions.

The NovaTec Epstein-Barr Virus (EBV) IgG-ELISA is intended for the qualitative determination of IgG class antibodies against Epstein-Barr virus. Samples are considered positive if the absorbance value is higher than 10% over the cut-off. The level of antibodies is expressed as NovaTec-Units = NTU.

ELISA-VIDITEST anti-EA is semiquantitative test. Samples with absorbances higher than 110% of the cut-off value are considered positive.

Statistical analysis

Descriptive statistics were used to characterize patient baseline characteristics. The Mann-Whitney *U*-test and Kruskal-Wallis test were used to compare antibody levels. Pearson's chi-square test was used to investigate the relationship between EBNA2 subtypes and LMP1

subtype and clinical and demographical parameters. Statistical significance was defined as $p < 0.05$.

Results

EBV DNA was detected in 52.70% of the patients with pharyngeal and laryngeal cancer and in 25.0% of controls ($p < 0.001$). In all patients with EBV DNA type 1 of the virus was detected. Epidemiological and demographical characteristics of the patients and controls are shown in Table 1.

The prevalence of EBV in patients group was significantly higher in males than in females ($p < 0.05$). No statistically significant differences were found between EBV infection and other demographical features, smoking and alcohol consumption (Table 2). The majority of patients (82.8%) were infected with wild type of EBV.

All the patients and controls were EBVCA IgM antibodies negative. IgG EBVCA antibodies were detected in 94.5% of patients, EBNA in 96.4% (Table 3). A statistically significant difference was observed in the prevalence of IgG EA antibodies in patients and controls (94.5% vs 22.5%). High level of EA was stated in 70% of cancer cases and in 0% of controls ($p < 0.05$). The level of IgG EBVCA and IgG EBNA antibodies was higher in the patients than in control group; however, the difference was not statistically significant.

Patients with an advanced stage of tumour development (T3-T4 stage) had a significantly higher level of EBNA antibodies than patients with T1-T2 stage ($p < 0.05$), while the level of EBVCA antibodies was the highest in patients with N1 stage. The level of EBVCA antibodies decreased with more advanced nodal stage ($p < 0.05$) (Table 4).

In patients infected with the wild-type of EBV, the level of anti-EBVCA was significantly higher than in cases with EBV type with deletion ($p < 0.05$). There was no relationship between EBV type on the basis of the

Table 3 EBVCA, EBNA and EA level in serum of patients and control group

| Antibodies level (IgG) | | Patients | | Controls | | p |
|------------------------|----------|----------|------|----------|------|--------|
| | | n | % | n | % | |
| EBVCA | negative | 6 | 5.5 | 0 | 0 | >0.05 |
| | low | 24 | 21.8 | 14 | 35.0 | |
| | high | 80 | 72.7 | 26 | 65.0 | |
| EBNA | negative | 4 | 3.6 | 0 | 0 | >0.05 |
| | low | 23 | 20.9 | 12 | 30.0 | |
| | high | 83 | 75.5 | 28 | 70.0 | |
| EA | negative | 6 | 5.5 | 31 | 77.5 | <0.05* |
| | low | 27 | 24.5 | 9 | 22.5 | |
| | high | 77 | 70.0 | 0 | 0 | |

*statistically significant

Table 4 Association between EBVCA, EBNA, EA antibodies level in patients serum and selected features (*p* value)

| Histological grading TN classification | EBVCA IgG | EBNA IgG | EA IgG |
|-------------------------------------------|---------------------|---------------------|--------|
| Histological grade G | 0.9139 | 0.2222 | 0.5587 |
| T stage | 0.6359 | 0.0174 ^a | 0.1759 |
| N stage | 0.0126 ^a | 0.5920 | 0.4201 |
| EBV type <i>LMP1 vs del-LMP1</i> | 0.0379 ^a | 0.4479 | 0.3622 |
| Location of cancer | 0.8875 | 0.9363 | 0.9485 |

^astatistically significant

sequence in *LMP1* gene, histological grading, and TN stage (Table 5).

Discussion

Two major types of EBV (EBV-1 and EBV-2) have been identified (a classification based on the EBNA2 gene sequence) and according to the researchers they differ in geographic distribution [6, 16]. According to some investigators, these genetic differences in EBV DNA sequence may be responsible for varied interactions with host cells, immunological response and cancer transformation, but the role of specific EBV types in the etiology of different cancers is not fully understood [17, 18]. EBV type 1 has a greater potential to transform B lymphocytes than EBV type 2 and is more common in Western and Asian countries, while EBV-2 is more frequently detected in Africa [16]. The results of our present and previous studies are consistent with the existing reports as type 1 EBV was detected in all examined patients [19]. Similar results were presented by Neves et al. [18], who demonstrated that 96.3% of the examined Portuguese individuals carried EBV type 1.

Table 5 Relationship between EBV type according to the *LMP1* gene sequence and histological grading, TN stage

| Histological grading TN classification | EBV <i>LMP1</i> | | EBV <i>del-LMP1</i> | | <i>p</i> |
|-------------------------------------------|-----------------|-------|---------------------|------|----------|
| | <i>n</i> | % | <i>n</i> | % | |
| G1 | 16 | 72.7 | 6 | 27.3 | 0.05113 |
| G2 | 30 | 88.2 | 4 | 11.8 | |
| G3 | 2 | 100.0 | 0 | 0 | |
| T1 | 6 | 100.0 | 0 | 0 | 0.2786 |
| T2 | 24 | 85.7 | 4 | 14.3 | |
| T3 | 14 | 87.5 | 2 | 12.5 | |
| T4 | 4 | 50.0 | 2 | 50.0 | |
| N0 | 26 | 86.7 | 4 | 13.3 | 0.6293 |
| N1 | 8 | 80.0 | 2 | 20.0 | |
| N2 | 12 | 85.7 | 2 | 14.3 | |
| N3 | 2 | 50.0 | 2 | 50.0 | |

As the disease progresses, EBV is activated into the replicative stage, in which major viral proteins are expressed [20, 21]. Patients with nasopharyngeal carcinoma show an elevated level of antibodies to several EBV antigens, including the viral capsid antigen (VCA), early antigen (EA) and EB nuclear antigen (EBNA) [22–25]. Traditional assays of EBV antibodies have been very useful in clinical diagnosis of nasopharyngeal cancer (NPC) [26].

Our study revealed that more than half of the patients were EBV positive but also the level of IgG EBV antibodies was higher in patients than in controls and it increased with the tumour development stage. Moreover, the difference in EBV DNA prevalence between patients and controls was statistically significant.

Serological testing was performed both in the patients and in the controls to clarify whether there is a relationship between pharyngeal and laryngeal cancer development and past EBV infection. Various studies revealed that IgG EBV antibodies were detected in a greater part of the examined patients and their level was higher in the patients with pharyngeal and laryngeal cancers than in controls [24, 27, 28]. Other researchers report IgG EBVCA antibodies detection in more than 60% of patients with laryngeal and pharyngeal cancers [29].

Our study did not reveal any significant difference between the presence and the level of EBV antibodies and demographical features of the patients, cancer location and histological grading of malignant lesions. However, patients with an advanced stage of tumour development (T3-T4 stage) had a significantly higher level of EBNA antibodies than patients with T1-T2 stage, while the level of EBVCA antibodies was the highest in patients with N1 stage. The more advanced nodal stage was, the more the level of these antibodies decreased.

The level of EA antibodies among our patients was higher than in control group. Our results are similar to other researchers' findings and may indicate a reactivation of latent EBV infection, because a high titer of EA antibodies can be seen in cases of reactivation of latent infection [25].

Statistically significant differences were observed by Chen et al. [30] in the scores/levels of six markers (EBV-IgG, VCA-IgG, EA-D p43-IgG, EA-IgG, EA-IgG + EBNA1-IgG, and EA-D p45-IgG) between NPC patients and healthy subjects. The lytic cycle of EBV is important and plays more active roles in oncogenesis [31].

Many authors show that EBV DNA load and IgA antibodies are more effective and useful in the clinical diagnosis and screening of NPC [32–34]. In our study only IgG antibodies were analysed. Immunoglobulins against viral proteins, including EA-IgG, VCA-IgA, and Rta-IgG, may be used as molecular biomarkers for predicting the prognosis of nasopharyngeal cancer. According to Tay et al. [24], EBV DNA load correlated with EA IgA serology

titers may be useful in detecting early NPC in screening studies.

EBV is transmitted via oral route and primary infection establishes a lifelong virus latent infection. The establishment of latent EBV infection in premalignant nasopharyngeal epithelial cells and the expression of latent viral genes are crucial features of NPC [14, 35].

Latent infection was divided into different subgroups due to specific viral proteins expression [12, 31, 34]. Nasopharyngeal carcinoma can display both latency type I and II EBV infections [36]. During type I latency EBNA1, EBER1 and 2, BamHI-A rightward transcripts (BART) are expressed, but type II latency can also express latent membrane protein 1 (*LMP1*). The oncogenic role of *LMP1* is well established. In nasopharyngeal carcinoma *LMP1* expression is associated with TNM stage and lymph node metastasis [37]. The EBV variant with a 30 bp deletion ((amino acids 346–355) includes part of C terminal activating region 2) isolated from an NPC tumor had a greater transforming activity than the reference *LMP1* [38]. The 30 bp deletion variant (*del-LMP1*) was first detected in EBV isolated from cell lines derived from NPC patients from Southern China [39]. Molecular studies demonstrated that a higher frequency of nasopharyngeal cancer detected in Asian population contains a variant of EBV *LMP1* gene with a 30-bp deletion (*del-LMP1*) [16, 34].

According to some researchers, EBV *del-LMP1* plays a key role in nasopharyngeal cancer development and might be detected at higher frequencies in NPC patients than in the general population. Other investigators, however, suggest that *del-LMP1* is only a geographic variation – it is more common in the Chinese population but not involved in the pathogenesis of NPC, as no association was found between the *del-LMP1* and NPC. Hadhri et al. [40] found that *del-LMP1* variant was significantly more frequent in NPC (71.42%) than in control biopsies (52%) in Tunisia. Tiwawech et al. [16] also reported that a significant association between the *del-LMP1* variant and NPC susceptibility was found in the Thai. Moreover, the frequency of *del-LMP1* in NPC patients was associated with the clinical stage of NPC [39].

Our study demonstrated that in the Polish population with oropharyngeal and laryngeal cancer *wild-type LMP1* was more frequent (83%). A limitation of our research is, however, small size of *del-LMP1* group, which makes statistical data comparing relationship between EBV type on the basis of the sequence in *LMP1* gene and histological grading or TN stage not sufficiently strong. Neves et al. [18] demonstrated that EBV-2 and *wt-LMP1* were associated with NPC in the Portuguese population. Their research performed in a similar ethnic group – Portuguese individuals – revealed no predominance of a specific *LMP1* variant as not only both variants

but also co-infection was common in this population. However, contrary to the Chinese population these authors found that the majority of NPC patients were *wt-LMP1*, which pointed to a differential geographic association of EBV-strains with NPC development.

Although the association between EBV infection and head and neck cancer was reported in various studies, the mechanism of malignancy development is still not clear. Understanding the role of the EBV latent genes expressed in pharyngeal and laryngeal cancers is crucial in determining the role of viral infection in the development and progression of cancer in this area.

Conclusions

Our results reveal that EBV DNA and a high level of antibodies, particularly EA, are most frequent and the wild type EBV is predominant in Polish patients with both pharyngeal and laryngeal carcinoma. However, further studies are necessary to clarify the role of Epstein-Barr virus in cancer development because genetic and epigenetic changes occur after EBV infection.

Abbreviations

BL: Burkitt's lymphoma; *del-LMP1*: deletion variant of latent membrane protein 1; EA: Early antigen; EBNA: Epstein-Barr nuclear antigen; EBV: Epstein-Barr virus; EBVCA: Epstein-Barr viral capsid antigen; HL: Hodgkin lymphoma; HNC: Head and neck cancer; *LMP1*: Latent membrane protein 1; NPC: Nasopharyngeal cancer; TN: Tumour, node; VCA: Viral capsid antigen; *wt-LMP1*: wild type latent membrane protein 1

Acknowledgements

Not applicable.

Funding

This study was supported by a Research Grant from the Medical University of Lublin, Lublin, Poland (DS 233).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

SF: Conceived the study, its design, data and clinical samples collection. MS-D: data analysis, manuscript preparation. BD: Statistical and data analysis. AB: carried out serological and molecular identification. MP-D: conceived the study, data analysis, coordination and help in drafting the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This research was approved by the Ethics Committee and is in accordance with the GCP regulations (no. KE-0254/133/2013).

All participants provided written informed consent to participate in this study according to forms required by the Local Ethics Committee.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Department of Virology, Medical University of Lublin, Lublin, Poland. ²Chair and Department of Conservative Dentistry with Endodontics, Medical University of Lublin, Lublin, Poland. ³Chair and Department of Public Health, Medical University of Lublin, Lublin, Poland.

Received: 8 March 2017 Accepted: 19 May 2017

Published online: 30 May 2017

References

- Hillbertz NS, Hirsch JM, Jalouli J, Jalouli MM, Sand L. Viral and molecular aspects of oral cancer. *Anticancer Res.* 2012;32(10):4201–12.
- Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer.* 2004;4(10):757–68.
- Macswen KF, Higgins CD, McAulay KA, Williams H, Harrison N, Swerdlow AJ, et al. Infectious mononucleosis in university students in the United Kingdom: evaluation of the clinical features and consequences of the disease. *Clin Infect Dis.* 2010;50(5):699–706.
- Tsang CM, Tsao SW. The role of Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma. *Virology.* 2015;53(2):107–21.
- Raab-Traub N. Nasopharyngeal carcinoma: an evolving role for the Epstein-Barr virus. *Curr Top Microbiol Immunol.* 2015;390:339–63.
- Deng Z, Uehara T, Maeda H, Hasegawa M, Matayoshi S, Kiyuna A, et al. Epstein-Barr virus and human papillomavirus infections and genotype distribution in head and neck cancers. *PLoS One.* 2014;9(11), e113702.
- Jalouli J, Jalouli MM, Sapkota D, Ibrahim SO, Larsson PA, Sand L. Human Papilloma virus, Herpes Simplex virus and Epstein Barr virus in oral squamous cell carcinoma from eight different countries. *Anticancer Res.* 2012;32:571–80.
- Alibek K, Kakpenova A, Baiken Y. Role of infectious agents in the carcinogenesis of brain and head and neck cancers. *Infect Agent Cancer.* 2013;8(1):7.
- Khan G, Hashim MJ. Global burden of deaths from Epstein-Barr virus attributable malignancies 1990–2010. *Infect Agent Cancer.* 2014;9(1):38.
- Gandini S, Negri E, Boffetta P, La Vecchia C, Boyle P. Mouthwash and oral cancer risk quantitative meta-analysis of epidemiologic studies. *Ann Agric Environ Med.* 2012;19(2):173–80.
- Sand L, Jalouli J. Viruses and oral cancer. Is there a link? *Microbes Infect.* 2014;16(5):371–8.
- Bornkamm GW. Epstein-Barr virus and the pathogenesis of Burkitt's lymphoma: more questions than answers. *Int J Cancer.* 2009;124(8):1745–55.
- Shield KD, Ferlay J, Jemal A, Sankaranarayanan R, Chaturvedi AK, Bray F, et al. The global incidence of lip, oral cavity, and pharyngeal cancers by subsite in 2012. *CA Cancer J Clin.* 2017;67(1):51–64.
- Young LS, Dawson CW. Epstein-Barr virus and nasopharyngeal carcinoma. *Chin J Cancer.* 2014;33(12):581–90.
- Kis A, Fehér E, Gáll T, Tar I, Boda R, Tóth ED, et al. Epstein-Barr virus prevalence in oral squamous cell cancer and in potentially malignant oral disorders in an eastern Hungarian population. *Eur J OralSci.* 2009;117(5):536–40.
- Tiwawech D, Srivatanakul P, Karalak A, Ishida T. Association between EBNA2 and LMP1 subtypes of Epstein-Barr virus and nasopharyngeal carcinoma in Thais. *J Clin Virol.* 2008;42(1):1–6.
- IARC monographs on the evaluation of carcinogenic risks to humans. A review of human carcinogens. Biological agents. Lyon: World Health Organization; 2012. p. 255.
- Neves M, Marinho-Dias J, Ribeiro J, Esteves M, Maltez E, Baldaque I, et al. Characterization of Epstein-Barr virus strains and LMP1-deletion variants in Portugal. *J Med Virol.* 2015;87(8):1382–8.
- Polz D, Podsiadło Ł, Stec A, Polz-Dacewicz M. Prevalence of EBV genotypes in Polish, Taiwanese and Arabic healthy students and association between genotypes and 30-bp deletion in the LMP-1 gene phylogenetic analysis. *Pol J Microbiol.* 2014;63(1):105–9.
- Makielski KR, Lee D, Lorenz LD, Nawandar DM, Chiu YF, Kenney SC, et al. Human papillomavirus promotes Epstein-Barr virus maintenance and lytic reactivation in immortalized oral keratinocytes. *Virology.* 2016;495:52–62.
- Ji MF, Huang QH, Yu X, Liu Z, Li X, Zhang LF, et al. Evaluation of plasma Epstein-Barr virus DNA load to distinguish nasopharyngeal carcinoma patients from healthy high-risk populations in Southern China. *Cancer.* 2014; 120(9):1353–60.
- Abdulmir AS, Hafidh RR, Abu Bakar F, Abbas K. Novel Epstein-Barr virus immunoglobulin G-based approach for the specific detection of nasopharyngeal carcinoma. *Am J Otolaryngol.* 2010;31(6):410–7.
- Linde A. Diagnosis of Epstein-Barr virus-related diseases. *Scand J Infect Dis Suppl.* 1996;100:83–8.
- Tay JK, Chan SH, Lim CM, Siow CH, Goh HL, Loh KS. The role of Epstein-Barr Virus DNA load and serology as screening tools for nasopharyngeal carcinoma. *Otolaryngol Head Neck Surg.* 2016;155(2):274–80.
- De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: problems and solutions. *World J Virol.* 2012;1(1):31–43.
- Chang KP, Hsu CL, Chang YL, Tsang NM, Chen CK, Lee TJ, et al. Complementary serum test of antibodies to Epstein-Barr virus nuclear antigen-1 and early antigen: a possible alternative for primary screening of nasopharyngeal carcinoma. *Oral Oncol.* 2008;44(8):784–92.
- Chien YC, Chen JY, Liu MY, Yang HI, Hsu MM, Chen CJ, et al. Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *N Engl J Med.* 2001;345(26):1877–82.
- Coghill AE, Hsu WL, Pfeiffer RM, Juwana H, Yu KJ, Lou PJ, et al. Epstein-Barr virus serology as a potential screening marker for nasopharyngeal carcinoma among high-risk individuals from multiplex families in Taiwan. *Cancer Epidemiol Biomarkers Prev.* 2014;23(7):1213–9.
- Roy A, Dey S, Chatterjee R. Prevalence of serum IgG and IgM antibodies against Epstein-Barr virus capsid antigen in Indian patients with respiratory tract carcinomas. *Neoplasma.* 1994;41(1):29–33.
- Chen H, Luo YL, Zhang L, Tian LZ, Feng ZT, Liu WL. EA-D p45-IgG as a potential biomarker for nasopharyngeal carcinoma diagnosis. *Asian Pac J Cancer Prev.* 2013;14(12):7433–8.
- Murata T. Regulation of Epstein-Barr virus reactivation from latency. *Microbiol Immunol.* 2014;58(6):307–17.
- Li Y, Wang K, Yin SK, Zheng HL, Min DL. Expression of Epstein-Barr virus antibodies EA-IgG, Rta-IgG, and VCA-IgA in nasopharyngeal carcinoma and their use in a combined diagnostic assay. *Genet Mol Res.* 2016;15(1).
- Peng YH, Xu YW, Huang LS, Zhai TT, Dai LH, Qiu SQ, et al. Autoantibody signatures combined with Epstein-Barr virus capsid antigen-IgA as a biomarker panel for the detection of nasopharyngeal carcinoma. *Cancer Prev Res (Phila).* 2015;8(8):729–36.
- Kikuchi K, Noguchi Y, de Rivera MW, Hoshino M, Sakashita H, Yamada T, et al. Detection of Epstein-Barr virus genome and latent infection gene expression in normal epithelia, epithelial dysplasia, and squamous cell carcinoma of the oral cavity. *Tumour Biol.* 2016;37(3):3389–404.
- Kang MS, Kieff E. Epstein-Barr virus latent genes. *Exp Mol Med.* 2015;47:e131.
- Frappier L. Role of EBNA1 in NPC tumorigenesis. *Semin Cancer Biol.* 2012; 22(2):154–61.
- Wang A, Zhang W, Jin M, Zhang J, Li S, Tong F, Zhou Y. Differential expression of EBV proteins LMP1 and BHRF1 in EBV-associated gastric and nasopharyngeal cancer tissues. *Mol Med Rep.* 2016;13(5):4151–8.
- Tzellos S, Farrell PJ. Epstein-Barr virus sequence variation – biology and disease. *Pathogens.* 2012;1(2):156–74.
- Ai J, Xie Z, Liu C, Huang Z, Xu J. Analysis of EBNA-1 and LMP-1 variants in diseases associated with EBV infection in Chinese children. *Virology.* 2012;9:13.
- Hadhri-Guiga B, Khabir AM, Mokdad-Gargouri R, Ghorbel AM, Drira M, Daoud J, Frikha M, et al. Various 30 and 69 bp deletion variants of the Epstein-Barr virus LMP1 may arise by homologous recombination in nasopharyngeal carcinoma of Tunisian patients. *Virus Res.* 2006;115(1):24–30.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

