EPSTEIN-BARR VIRUS (EBV) AND ITS ASSOCIATION WITH NASOPHARYNGEAL CARCINOMA (NPC): A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: NPC has viral, environmental, and genetic components to its etiology. The involvement of Epstein-Barr Virus (EBV) in NPC has been postulated since the year 1966, when NPC patients were found to express antibodies against an antigen later identified as EBV (Old LJ, Boyse EA and Oettgen HF 1966). EBV infection have been associated with development of several malignancies, including nasopharyngeal carcinoma (IARC; 1997). The latency period usually lasts for several decades before the onset of NPC (Ellen T, Chang and Hans-Olov Adami 2006). Following the discovery of EBV, various studies were undertaken to find an association between the virus and NPC. A systematic review was performed to evaluate the available literature on the relationship between EBV and NPC.

Data Sources: Systematic review of primary studies identified through MEDLINE, DynaMed, and Cochrane Database. Systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. MEDLINE, EMBASE, and Cochrane databases were queried for English language studies published between 2001 and 2016.

Results: The initial database from the search performed identified 68 articles, which were screened for relevance to the assessment of EBV and its association with NPC. In addition to these 68 articles, there were 4 other articles, which were identified through other sources. Manual searching of reference lists of the 48 full text articles yielded no additional eligible studies. In total, 49 articles were included for the systematic review.

Conclusion: The findings of this systematic review suggest an association between Epstein - Barr virus and nasopharyngeal carcinoma. These findings are consistent with the current understanding of the role of EBV as a viral agent in association with the occurrence of NPC.

Keywords: Epstein - Barr virus, EBV, Nasopharyngeal Carcinoma, NPC
1.0 Introduction

There were an estimated 84,400 incident cases of NPC and 51,600 deaths in 2008, representing about 0.7% of the global cancer burden (IARC 2012). NPC is highly prevalent in the Southeast Asia. In Peninsular Malaysia, NPC is the fifth most common cancers overall and third most common among men (Ministry of Health Malaysia 2006).

NPC has viral, environmental, and genetic components to its etiology. The involvement of Epstein - Barr virus (EBV) in NPC has been postulated since the year 1966, when NPC patients were found to express antibodies against an antigen later identified as EBV (Old LJ, Boyse EA and Oettgen HF 1966). Various serological tests for antibodies against EBV antigens have been developed to detect NPC as subsequent studies have demonstrated higher anti-EBV antibodies observed among NPC patients compared to controls (Henle W, Henle G and Ho HC 1970). EBV infection have been associated with development of several malignancies, including nasopharyngeal carcinoma (IARC; 1997). The latency period usually lasts for several decades before the onset of NPC (Ellen T, Chang and Hans-Olov Adami 2006). Following the discovery of EBV, various studies were undertaken to find an association between the virus and NPC.

The disease burden of Epstein - Barr virus (EBV) associated nasopharyngeal carcinoma (NPC) is prevalently high, particularly in the South East Asia and Southern China region (Razak AR, et al. 2010). Major risk factors for NPC are ubiquitous environmental agents that interact with a genetic background of susceptibility to result in adverse immune control of EBV infection, ultimately leading to NPC (Ellen T, Chang and Hans-Olov Adami 2006).

At present, the prognostication of NPC has been accomplished mainly by a variety of clinical and radiological parameters. It is further been shown that the serial analysis of circulating EBV DNA offers a powerful method for monitoring the progress of these patients (Lo YM, et al. 1999). A systematic review was performed to evaluate the available literature on the relationship between EBV and NPC.

2.0 Materials and Methods

A comprehensive review of the English-language literature was performed from the MEDLINE, DynaMed, and Cochrane Database of Systematic Reviews using the EBSCOhost portal. Search criteria included all occurrences in the title or abstract of the terms nasopharynx, nasopharyngeal carcinoma, and Epstein - Barr virus or EBV, published between 2001 and 2016. Prospective or respective case-control or cohort studies regarding the association between EBV and NPC were included in this review.

Inclusion criteria for the literature search were defined using the Population, Intervention, Control, Outcome, Study Design (PICOS; Table 1) approach. Systematic search was performed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; Figure 1).
Duplicate records were removed. The abstract of every citation was then screened for relevance to the Epstein-Barr virus and its association with nasopharyngeal carcinoma. Irrelevant citations and case reports were excluded. The full text of remaining citations was obtained along with additional records from the reference lists of the published articles. The full text articles were reviewed and non-eligible studies were excluded. Data gathered from full text articles included criteria for patient selection, study design, number of cases of NPC, history of NPC, and WHO histological classification. The risk of bias was assessed at the study level by examining each study for design, author’s stated purpose for the study, and source of patient data collection.

**Table 1:** Population, Intervention, Control, Outcome, Study Design (PICOS) Inclusion Criteria.

<table>
<thead>
<tr>
<th>PICOS</th>
<th>Description</th>
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<tbody>
<tr>
<td>Population</td>
<td>Adult (&gt; 18 years old) men and women</td>
</tr>
<tr>
<td>Intervention</td>
<td>Adults with a history of nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>Control</td>
<td>Adults without a history of nasopharyngeal carcinoma</td>
</tr>
<tr>
<td>Outcomes</td>
<td>The role of EBV in the carcinogenesis of NPC</td>
</tr>
<tr>
<td>Study design</td>
<td>Randomized controlled trials, Non-randomized controlled trials, Retrospective or prospective cohort studies, Cross sectional studies</td>
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</tbody>
</table>

**Figure 1:** Preferred Reporting Items for Systematic Reviews and Meta-Analyses
3.0 Discussion

3.1 Epstein-Barr virus as a carcinogen

In 1964, EBV was identified in tumor tissue from a patient who had African Burkitt lymphoma, a fatal malignancy of the B lymphocyte (Epstein MA, Achong BG and Barr YM 1964). It was described as a member of the Lymphocryptovirus genus viruses that are closely related members of herpesvirus family with the EBV genome exceeding 172 kb pairs of linear double-stranded DNA. EBV was also the first herpesvirus to have its genome completely cloned and sequenced (Baer R, Bankier AT and Biggin MD 1984). During growth transformation, the virus does not replicate and produce progeny virions, but rather is replicated by the host DNA polymerase as an extra chromosomal episome (Raab-Traub N 2002).

EBV is a γ-herpes virus present in over 90% of adults worldwide (Henle W, HEnle G and Ho HC 1970). Though the infection is lifelong, it usually remains harmless unless the balance between host and virus is altered. Diseases associated with EBV include those of lymphocytic origin (infectious mononucleosis, Hodgkin’s disease, and Burkitt’s lymphoma) and epithelial origin (Nasopharyngeal Carcinoma, oral hairy leukoplakia, and undifferentiated gastric carcinoma) (Macsween K and Crawford D 2003). At least 95% of NPC tumors are EBV associated (Chou Josephine, Lin and You 2008). Additionally, the severity of EBV infection varies with carcinoma type, with undifferentiated carcinomas (Type 3 WHO) having the highest EBV titers (Wei W and Sham J 2005).

3.2 Role of EBV in the carcinogenesis of NPC

The association between EBV and NPC was first described by Old et al in 1996 using in situ hybridization and the anticomplement immunofluorescent (ACIF) assay. Subsequent studies by others demonstrated the expression of EBV latent genes namely Epstein–Barr virus nuclear antigen (EBNA), latent membrane protein-1 (LMP-1), LMP-2, and EBV encoded small RNAs (EBER) in NPC cells confirming the infection of tumor cells by EBV (Baumforth KR, Young LS and Flavel KJ 1999). Studies have proposed that EBV plays a critical role in transforming nasopharyngeal epithelial cells into invasive cancer (Lo KW, To KF and Huang DP 2004). It was also found that EBV-positive tumors grew faster than EBV negative tumors, and also had clonal EBV terminal repeat sequences (Wu HC, Lin YJ and Lee JJ 2003).

The presence of monoclonal EBV episomes in NPC indicates that viral infection precedes the clonal expansion of malignant cells (Raab-Traub; 2002). However, epithelial infection may not be the initiating event in virus-associated carcinogenesis, as tonsils from patients with infectious mononucleosis (IM) and normal nasopharyngeal biopsies from individuals at high risk of developing NPC lack evidence of epithelial EBV infection (Young; 2004).

EBV has tumorigenic potential due to a unique set of latent genes: latent membrane proteins (LMP1, LMP2A, and LMP2B) and EBV-determined nuclear antigens (EBNA1 and EBNA2) are the proteins predominantly expressed in NPC (Brooks L, et al. 1992). LMP1 is the principal oncogene of NPC and it is required for cell immortalization and is present in 80% to 90% of NPC tumors (Wang D, Liebowitz D and Kieff E 1985). The LMP1 molecule includes 6 transmembrane domains and a carboxyl terminus containing 2 signalling domains called C-
terminal activating regions 1 and 2 (CTAR 1 and 2) (Chou Josephine, Lin and You 2008). The transmembrane domains allow LMP1 to associate with the host membrane, whereas the CTAR regions directly activate a number of signaling pathways including nuclear factor κ-B (NF-κB), mitogen-activated protein (MAP) kinases, and phosphoinositol-3-kinase (PI3K) (Mainou BA and Raab-Traub N 2006).

Although the basic role of LMP1 is to prevent apoptosis, it has other important functions in cancer development (Chou Josephine, Lin and You 2008). LMP1-positive cells have greater mobility, leading to higher metastatic potential and faster disease progression (Ozyar E, et al. 2004). LMP1 is also involved in suppressing immunogenic responses against NPC; for example, LMP1 has intrinsic T-cell inhibitory properties and mediates downregulation of CD99, which is an important component of the anti-NPC immune response (Chou Josephine, Lin and You 2008). The importance of LMP1 in tumorigenesis is illustrated by numerous studies that show the inhibition of LMP1 results in increased tumor cell sensitivity to chemotherapy (Mei YP, Zhou JM and Wang Y 2007).

LMP2A plays a role by downregulation of the NF-κB transcription factor and can decrease LMP1 expression (Steward S, Dawson CW and Takada K 2004). Additionally, LMP2A expression also causes NPC cells to become migratory and invasive (Petgel DM, et al. 2005). EBNA1 is an unusual protein that binds the EBV genome to host chromosomes, and thus mediates equal partitioning of viral DNA into daughter cells during cell division and may play a role in immune evasion (Chou Josephine, Lin and You 2008). EBNA2 may be involved in the transactivation of LMP1 (Wang F, et al. 1990).

Intriguingly, expression of EBV early antigen (EA) is positively correlated with the consumption of salted and preserved food, suggesting that development of EBV-positive NPC could be related to dietary habits, and provides another link to the epidemiological studies with NPC (Shao YM, Poirier S and Ohshima H 1988).

The Epstein-Barr Virus persists latently in over 90% of the world population (Rickinson AB and Kieff E 2001). It was reported that 80% of children in Hong Kong have been infected by EBV by 6 years, with almost 100% have seroconverted by the age of 10 years (Kangro HO, et al. 1994).

An elevated level of IgG and IgA antibody titers to the EBV viral capsid antigen (VCA) IgA and early antigen (EA) were observed among NPC patients, as well as increased IgG against the latent viral nuclear antigens 1 and 2 (EBNA-1, EBNA-2) and neutralizing antibodies against EBV-specific DNase (Ellen T, Chang and Hans-Olov Adami 2006). Interestingly, these antibody titers, especially of IgA, precede tumor development by several years and are correlated with tumor burden, remission, and recurrence (Chien YC, Chen JY and Liu MY 2001).

Based on these patterns, antibody against viral capsid antigen (VCA) was established as the basis of a screening test for NPC in high-risk populations, particularly in combination with anti-EBV DNase antibodies (Ellen T, Chang and Hans-Olov Adami 2006). This further led to the detection of circulating cell-free EBV DNA at higher proportion among NPC patients compared to controls (Lin JC, Wang WY and Chen KY 2004). The levels also correlate positively with disease stage and prognosis (Chan AT, Lo YM and Zee B 2002).
Clonal EBV has also been detected in severe dysplasia or carcinoma in situ of the nasopharynx indicating a role for the virus in the early stages of tumor progression (Ellen T, Chang and Hans-Olov Adami 2006). Compared with the prototype B95.8 EBV strain, consistent nucleotide variation in the amino terminus of the oncogenic viral latent membrane protein 1 (LMP1), including the loss of a XhoI restriction site, has been detected in EBV in NPC tumors from different ethnicity such as southern and northern Chinese, Malays, Alaska natives, and some U.S. Caucasians, but not North Africans.

Other types of sequence variation in the LMP1 carboxyl terminus including the number of copies of a 33-bp repeat element, a 15-bp insertion in the third repeat element, and a 30-bp deletion in the carboxyl terminus have been detected among Chinese NPC patients (Tan El, Peh SC and Sam CK 2003). The collective evidence strongly indicates a causal role of EBV in the development of NPC typically at high incidence areas (Edwards RH, et al. 2004).

However, EBV alone is not a sufficient cause of NPC, because virtually all adults worldwide are infected with the virus, yet only a small proportion of individuals develop NPC (Ellen T, Chang and Hans-Olov Adami 2006). Therefore, it is clear that other factors such as the environmental or genetic cofactors also play a role towards development of NPC.

### 3.3 EBV and its association with histological classification of NPC

In 1978, the World Health Organization (WHO) classified NPC into three different histologic types. This classification was revised later in 1991 (S-H.I.Ou, et al. 2007). Type 1, keratinizing squamous carcinoma, is characterized by well-differentiated cells that produce keratin, while Type 2 non-keratinizing squamous carcinoma, varies in cell differentiation (from mature to anaplastic cells) but does not produce keratin, and Type 3 which is also non-keratinizing, is less differentiated, with highly variable cell types (clear cell, spindle cell, anaplastic) (Shanmugaratnam K and Sobin LH ; 1993).

Types 2 and 3 NPC are Epstein–Barr virus (EBV) associated and have better prognoses than type 1, and EBV infection is generally absent in type 1, especially in non-endemic areas (Marks JE, Philips JL and Menck HR 1998). In the endemic areas, WHO type 3 is commonly seen, while the type 2 usually comprises less than 5% among these population (Shanmugaratnam K and Sobin LH ;1993).

In comparing survival between patients with keratinizing carcinoma, and non-keratinizing carcinoma, the 5-year survival rate is significantly better for non-keratinizing carcinoma than keratinizing squamous cell carcinoma of the nasopharynx (51% versus 6%) (Reddy SP, Raslan WF and Gooeratne S 2005). In addition, non-keratinizing carcinomas are generally associated with EBV positivity and EBV positivity in turn has been shown to be associated with improved survival (Shi W, Pataki I and MacMilan C 2002). However, most of the population-based studies in NPC are from endemic countries where the ethnic makeup of the population was fairly homogeneous and the undifferentiated non-keratinizing type histology is the predominant histology (S-H.I.Ou, et al. 2007).

The study conducted by S.-H.I.Ou et al (2007) also confirmed that WHO histology is an independent prognostic factor, in line with other studies (Burt RD et al; 1992). In addition, Chinese ethnicity was not only found to be another independent factor, but also a favorable prognostic factor for survival by multivariate analyses.
The observed improved survival of Chinese NPC patients however cannot simply be explained by the higher proportion of favorable non-keratinizing carcinoma. The Epstein-Barr virus (EBV) plays an important role in the pathogenesis of non-keratinizing carcinoma, whereas keratinizing squamous cell carcinoma histological type is the least related to EBV infection among the three different histologies (Niedobitek G, Hansmann ML and Herbst H 1991).

3.4 Quantification of EBV among NPC patients

In recent studies, it has been demonstrated that the cell-free EBV DNA can be found in the plasma and serum of NPC patients (Mutirangura A, et al. 1998). This has opened up new possibilities for monitoring NPC, which is a common type of malignancy among the population in south China and South East Asia. It has also been shown that serial analysis of circulating EBV DNA could be useful method to monitor progress of NPC patients (Lo YMD, Leung SF, et al. 2000). One study have explored the prognostic implication of the plasma/serum EBV DNA level at presentation, and concluded that the plasma/serum EBV DNA concentration is an independent prognostic factor for NPC (Dennis, Chan and YS 2000).

This study further noted that this prognostic effect could be seen with regard to both recurrence and metastasis within the first year after treatment and ultimate survival from the disease. Interestingly, it was also stated that for prognostication within the first year after treatment, plasma EBV DNA level was a more powerful prognosticator than disease stage.

The biological explanation for this scenario could be due to the EBV DNA concentration in plasma/serum provides a measure of tumour load that is present and potentially, this provides more accurate reflection of tumor burden than conventional staging systems (Dennis, Chan and YS 2000). It was also recommended in this study that the plasma/serum EBV DNA level could be incorporated into future revision of the staging system for NPC as it would potentially enhance the precision of disease prognostication.

Besides plasma EBV DNA measurement, salivary EBV DNA has also emerged as a valuable tool to monitor EBV DNA level among NPC patients. In a study conducted by (Edmond et al; 2011, it observed that the EBV DNA level of post-treatment saliva samples was significantly higher than the pre-treatment, and there is a trend that patients with advanced stage showed a higher EBV DNA level than patients with early stage disease. Future studies can test the sensitivity and specificity of these two methods.

4.0 Conclusion

The findings of this systematic review suggest an association between Epstein - Barr virus and nasopharyngeal carcinoma. These findings are consistent with the current understanding of the role of EBV as a viral agent in association with the occurrence of NPC. Prospective cohort studies would better establish the time to diagnosis from exposure. Quantification of EBV as a biomarker could be a useful method to monitor the disease status among NPC patients.
References


