**KESANS** : **International Journal Of Health and Science**
**e-ISSN : 2808-7178, p-ISSN : 2808-7380**
**Web :** http://kesans.rifainstitute.com/index.php/kesans/index

# Preliminary Study On Latent Membrane Protein-1 (LMP-1) And Bcl-2 Associated X (BAX) In Exosomes Of Patients With Nasopharingeal Cancer In NTB Provincial Hospital

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**Article Information**
*Accepted :* **08 Nov 2021**
*Submitted :* **12 Nov 2021**
*Online Publish :***20 Nov 2021**

**Abstract**

*Nasopharyngeal cancer is one of the most common types of cancer in Indonesia, especially head and neck cancer. One of the etiology of this cancer is infection with Epstein-Barr virus. Early diagnosis is difficult to establish because the early signs and symptoms of nasopharyngeal carcinoma are not specific. Examination of latent membrane protein-1 (LMP1) oncogene expression and BCL2-associated X (BAX) gene expression proved to be useful in the identification of nasopharyngeal cancer. LMP1 oncogene is an oncogene that functions as a tumor necrosis factor receptor (TNFR), so that apoptosis does not occur and promotes invasion and metastasis. The BAX gene is a pro-apoptotic gene that inhibits cancer development, but its regulation will be downregulated by LMP1 because LMP can protect B-cells from apoptosis. LMP1 oncogene is an oncogene that functions as a tumor necrosis factor receptor (TNFR), so that apoptosis does not occur and promotes invasion and metastasis. The BAX gene is a pro-apoptotic gene that inhibits cancer development, but its regulation will be downregulated by LMP1 because LMP can protect B-cells from apoptosis*

***Keywords : Nasopharyngeal Cancer; BCL2-Associated X; Latent Membrane Protein-1;***

## Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor that appears in the nasopharynx (the area above the throat and behind the nose) (Kemenkes RI, 2017; (Soepardi, Iskandar, Bashiruddin, & Restuti, 2012); (Salehiniya, Mohammadian, Mohammadian-Hafshejani, & Mahdavifar, 2018). The geographical distribution of nasopharyngeal carcinoma is not balanced. 81% of new cases occurred in Asia and 9% occurred in Africa; the rest were reported in other parts of the world. The standard incidence rate of nasopharyngeal cancer in the world is 1.2 per 100,000 people (1.7 per 100,000 men and 0.7 per 100,000 women). In Indonesia, the incidence rate is relatively high, compared with the world, at least 5.7/100,000 for men and 1.9/100,000 for women incidence rates of 1.9 per 100,000 in men and 0.8 per 100,000 in women, has been reported.

EBV infection associated with the pathogenesis of NPC has been attributed to the role of exosomes. Exosomes are defined as membrane-bound vesicles that form within multivesicular bodies (MVBs) or late endosomes secreted from cells (Ahmed, Philip, Tariq, & Khan, 2014). EBV persistently infects NPC cells as a latency pattern II expressing episomal anchoring protein *Epstein-Barr virus nuclear antigen 1* (EBNA1), *EBV-encoded small RNA* (EBERs), BARTs, *BamH I-A rightward frame-1* (BARF1), *Latent membrane protein-1* (LMP1), and LMP2A. Expression of this protein can be used to confirm the presence of EBV infection in tumor cells (Gurning, Lubis, & Delyuzar, 2015).

LMP1, which encodes the EBV oncogene, exhibits oncogenic potential and induces processes leading to the transformation of B-cell and epithelial-cell tumors. LMP1 encodes the EBV oncogene, which allows the virus to exist inside immune system cells for a long time. LMP1 is also known to induce cell cycle disruption and genomic instability in NPC cells. Together with BCL-2, LMP1 inhibits apoptosis so that it is anti-apoptotic (Sudiono & Hassan, 2013); (Richardo et al., 2020, p. 2441). The *BCL2-associated X* (BAX) gene encodes the most important pro-apoptotic gene of the BCL-2 family. BCL-2 which interacts with cell death mediators (BIM) which is a proapoptotic protein in BAX-induced mitochondrial apoptosis, is downregulated by EBV miR-BARTs (Richardo et al., 2020)

## Anatomy of the Nasopharynx

Nasopharynx is a cuboidal space or cavity located behind the nose. The boundaries of the nasopharyngeal cavity, on the front are the choana (nares posterior). Above, which is also the roof is the base of the cranium. Behind is the mucosal tissue in front of the cervical vertebrae. Below is the pharyngeal isthmus and soft palate, and the other boundary is the two lateral sides (Soepardi et al., 2012)

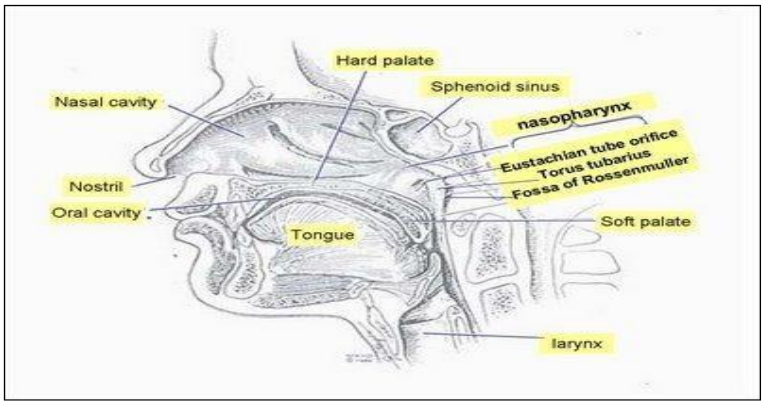


Figure 1.
Anatomy of the Nose and Nasopharyngeal Side View (Soepardi et al., 2012)

## Carcinoma Nasopharyngeal

### Definition

Carcinoma is a malignant tumor arising in the epithelium lining the space behind the nose (nasopharynx), which shows evidence of mild microscopic or ultrastructural squamous differentiation. These tumors originate from the lateral wall of the nasopharynx (Russenmuller fossa) and can spread into or out of the nasopharynx to the lateral, posterosuperior walls, skull base, palate, nasal cavity, and oropharynx and metastasize to cervical lymph nodes. The most common type of NPC is squamous cell malignancy (Primadina & Imanto, 2017); Ministry of Health, 2017; Snell, 2012).

### Epidemiology

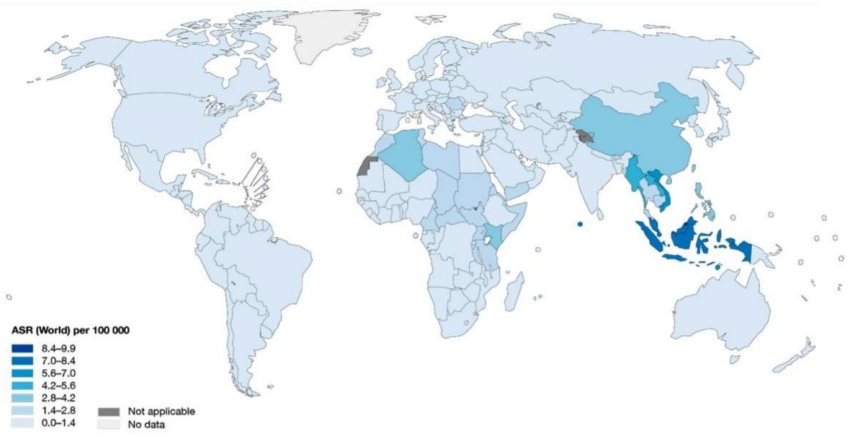


Figure 2.
Global distribution of nasopharyngeal carcinoma ((Richardo et al., 2020)

In 2018, global estimates of cancer incidence worldwide have been reported to be around 18.1 million cases, with 129,079 of these cases being NPC which estimates the age standard rate (ASR) of 1.5 per 100,000 people. In the same year, a total of 72,987 deaths from NPC were reported, accounting for 0.8% of the total cancer deaths. Comparing the incidence with other types, NPC can be considered an uncommon cancer, and its distribution worldwide is very unequal. NPC is a rare cancer in the United States (ASR 0.45) and most European countries (ASR 0.44). However, it is much more common in Asia, especially in Southeast Asian (ASR 5.0) and East Asian (ASR 2.7) countries where 27% and 50% of total NPC cases have been reported in the two regions, respectively (Richardo, *et al. al*., 2020).

**Etiology**

It is almost certain that the cause of nasopharyngeal carcinoma is the Epstein-Barr virus, because all patients with nasopharyngeal carcinoma have high titers of anti-EB virus. This titer is higher than the titer of healthy people, patients with malignant tumors of the neck and other heads. However, this virus is not the only factor, because there are many other factors that influence the possibility of this tumor, such as (Soepardi, 2012; Anggidian, 2017).

**a. Genetic Factors**

Changes lead to the proliferation of cancer cells in an uncontrolled manner. These changes are largely due to mutations, breakage of chromosomes, and loss of somatic cells. Several studies have stated that HLA (*Human Leukocyte Antigen*) plays an important role in the incidence of NPC.

**b. Environmental factor**

Several habits/foods have been reported to be associated with an increased risk of NPC. Consumption of salted fish and preserved foods containing volatile nitrosamines is an important carcinogenic factor associated with NPC. Other factors that are thought to play a role in the occurrence of nasopharyngeal cancer are dust, cigarette smoke, chemical fumes, firewood smoke, incense smoke, industrial wood powder, and traditional medicines, but a clear relationship between these substances and nasopharyngeal cancer has not been explained.

**c. Epstein-Barr Factor**

Virus Epstein-Barr virus is a carcinogen that causes several malignancies in humans, including NPC. Serum IgA antibodies, namely *viral capsid antigen* (VCA) and *early antigen* (EA) were significantly associated with NPC.

**Clinical Manifestations**

In the early stages, these tumors are difficult to identify. If there is a mass in the neck, neurological disease, or distant metastases, the patient is usually in an advanced stage. Symptoms include nasal congestion, nosebleeds, tinnitus, ear fullness, ear pain, diplopia, trigeminal neuralgia and neck masses (Kemenkes RI, 2017).

**Diagnosis**

The diagnosis of nasopharyngeal cancer is carried out through history taking, physical examination and supporting examinations.

**1) History**

Symptoms that are usually complained of are nasal symptoms, ear symptoms, eye and nerve symptoms, and metastatic/neck symptoms. Symptoms include nasal congestion, bloody mucus, tinnitus, ear fullness, ear pain, diplopia and trigeminal neuralgia (nerves III, IV, V, VI) and a lump in the neck (Kemenkes RI, 2017).

**2) Physical**

Examination General and local status examination. Examination of the nasopharynx can be performed with posterior rhinoscopy and nasopharyngoscope. On physical examination revealed a neck mass, cranial nerve palsy, and a mass in the nasopharynx on examination with a nasopharyngoscope (Soepardi, 2012; Kemenkes RI, 2017)

**3) Support Examination**

- Radiological examination

Coronary, axial and sagittal sections of the CT scan/MRI of the nasopharynx in the form of radiological examination (without or with contrast) help observe the primary tumor and spread to surrounding tissues and lymph nodes. For distant metastases, chest X-rays, bone scans, and abdominal ultrasound are required (Kemenkes RI, 2017).

- Anatomical Pathological Examination

Nasopharyngeal carcinoma was confirmed by pathological anatomic specimens and nasopharyngeal biopsy. The biopsy is performed using biopsy forceps inserted through the nose or mouth under posterior rhinoscopy or rigid/fiber nasopharyngoscopy. Reporting on the diagnosis of nasopharyngeal carcinoma based on WHO criteria, namely (Kemenkes RI, 2017):

1. Keratinized squamous cell carcinoma (WHO 1)
2. Carcinoma : No keratinized undifferentiated (WHO2) and not differentiate (WHO 3)
3. Squamous basaloid carcinoma

- Laboratory

Studies For nonkeratinizing or undifferentiated histology, consider testing for EBV in tumor and blood. Common ways to detect EBV in pathological specimens include in situ hybridization for *EBV-encoded RNA* (EBER) or immunohistochemical staining for *latent membrane protein* (LMP). Serum or plasma EBV DNA levels can be quantified using *polymerase chain reaction* (PCR) targeting the genomic sequence of EBV DNA such as BamHI-W, EBNA, or LMP; these tests vary in their sensitivity (Pastor, *et al*., 2018).

Table 1. TNM system in nasopharyngeal cancer based on AJCC 8th edition 2017 (Pastor et al., 2018).

| **Tumor (T)** | | **Lymph Nodes (N)** | | **Metastases (M)** | |
|---|---|---|---|---|---|
| T: | The Primary Tumor | N: | Enlargement of the KGB regional | M: | Distant metastases |
| T0: | No visible tumor, but positive EBV in the KGB neck | NX: | Enlargement lymph nodes can not be assessed | M0: | No distant metastases |
| T1s: | Carcinoma in situ | N0: | No KGB Enlargement | M1: | there is distant metastases |
| T1: | tumor confined to the nasopharynx, or tumor extends to the oropharynx and / or nasal cavity without the involvement of parafaring | N1: | metastasis KGB cervical unilateral and / or unilateral or bilateral metastases Retropharyngeal lymph node with the largest size 6 cm above the border caudal of the cricoid cartilage | | |
| T2: | Tumor extends to the region parapharyngeal and/or infiltrates into the pterygoid medial, pterygoid node lateral and/or prevertebral muscles. | N2: | Bilateral cervical lymphmetastases, with the largest size 6 cm above the caudal border of the cricoid cartilage. | | |

| | | | |
|---|---|---|---|
| T3: | Tumor invades bony structures and/or paranasal sinuses | N3: | Metastases lymph Bilateral, node largest size 6 cm or below the cricoid |
| T4: | Tumor with intracranial extension and/or involvement of the cranial nerves, infratemporal fossa,hypopharynx, orbit,gland parathyroid and/or infiltration to the lateral surface of the lateral pterygoid muscle. | | |

Table 2. Staging of nasopharyngeal cancer (Pastor et al., 2018)

| **Stadium** | **T** | **N** | **M** |
|---|---|---|---|
| Stadium 0 | T1s | N0 | M0 |
| Stadium 1 | T1 | N0 | M0 |
| Stadium II | T1 | N0, N1 | M0 |
| | T2 | N0, N1 | |
| Stadium III | T0, T1, T2 | N2 | M0 |
| | T3 | N0, N1, N2 | |
| Stadium IVA | T4 | N0, N1, N2 | M0 |
| | All T | N3 | |
| Stadium IVB | All T | All N | M1 |

**Prognosis**

As with most other neoplastic diseases, the strongest predictor of prognosis is the NPC stage. According to the *American Cancer Society*, the 5-year survival rate for patients with stage 0 is close to 100% and this is reduced to about 80% and 60% for stage I and II. For patients with stage III, IVA, this drops to between 30% and 40%. Patients with stage IVB, that is, distant metastatic disease, have a 5-year survival rate of 10%. The extent of the primary tumor should be assessed by MRI and/or CT scan (Petersson, 2015)

**Exosomes**

Exosomes are defined as membrane-bound vesicles that form within multivesicular bodies (MVBs) or late endosomes secreted from cells. The role of exosomes in viral pathogenesis has been studied several times, one of which is exosomes from nasopharyngeal carcinoma (NPC) infected with *Epstein Barr Virus* (EBV). These exosomes are known to contain *hypoxia-inducible factor-1a* (HIF1a) and *latent membrane protein*-1 (LMP-1) which contribute to tumor development and progression in NPC (Aga et al., 2014). NPC-derived exosomes also play a dual role in immune evasion to support and sustain tumor growth (Mrizak et al., 2015).

Exosomes are known to transfer proteins, DNA, mRNA, and miRNA from cell to cell to modulate diverse cellular processes (Schwab et al., 2015). Many EBV-*associated exosomes* were detected in virus-infected cells, so it is suspected that these exosomes can assist EBV in manipulating the tumor microenvironment to support tumor growth and survival. Exosomes are also known to "hijack" exosome pathways for the purpose of virus escape and evasion from the immune system (Ahmed et al., 2014). EBV-associated exosomes specifically package various viral components (e.g., LMP-1, LMP-2A, EBERs, viral RNA, miRNA) that can promote EBV infection (Schwab et al., 2015)

***Latent Membrane Protein*-1 (LMP1)**

EBV persistently infects NPC cells as a latency pattern II expressing episomal anchoring protein *Epstein-Barr virus nuclear antigen 1* (EBNA1), *EBV-encoded small RNA* (EBERs), BARTs, *BamH IA rightward frame-1* (BARF1), *latent membrane protein-1* (LMP1), and LMP2A. Expression of this protein can be used to confirm the presence of EBV infection in tumor cells (Gurning et al., 2015); (Richardo et al., 2020). LMP1 is one of the major EBV-encoded oncogenes associated with virus-mediated transformation. It is known that LMP1 is responsible for making an oncoprotein that functions as a constitutively active tumor necrosis factor receptor (TNFR) and acts as a major modulator in the pathogenesis and development of NPC, especially in invasion and metastasis.

LMP1 encodes the EBV oncogene, which allows the virus to exist inside immune system cells for a long time. This suggests that the tight regulation of LMP1 might reflect the co-evolution of oncogenesis, immune evasion, and viral pathogenesis in host cells. LMP1 is also known to induce cell cycle disruption and genomic instability in NPC cells. Together with BCL-2, LMP1 inhibits apoptosis so that it is anti-apoptotic (Sudiono & Hassan, 2013); (Richardo et al., 2020)

### *BCL2-associated X* (BAX)

The BCL-2 family has long been identified for its role in apoptosis. The initial discovery of BCL-2 in cases of B-cell lymphoma in the 1980s, identified a number of homologous proteins. Members of the BCL-2 family are grouped according to the BCL-2 (BH) homology domain and involvement in the regulation of apoptosis. The BH domain facilitates interaction of family members with each other, and may exhibit pro- or anti-apoptotic functions. Traditionally, these proteins have been categorized into one of three subfamilies namely anti-apoptotic, BH3-*only* (pro-apoptotic), and pore-forming or 'executioner' (pro-apoptotic) proteins. Subfamily categorization has traditionally been based on BH and transmembrane domains as well as status of anti- or pro-apoptotic function, and pore-forming ability. *BCL2-associated X* (BAX) belongs to the subfamily of pore-forming (pro-apoptotic) proteins (Warren, Wong-Brown, & Bowden, 2019)

The *BCL2-associated X* (BAX) gene encodes the most important pro-apoptotic gene of the BCL-2 family. BCL-2 which interacts with cell death mediators (BIM) which is a proapoptotic protein in BAX-induced mitochondrial apoptosis, is downregulated by EBV miR-BARTs (Richardo et al., 2020). BAX expression is also associated with tumor development and haematological malignancies due to dysregulation of the apoptotic process. The apparent downregulation of BAX may be due to *Latent Membrane Protein-1* (LMP1) EBV. This antiapoptotic protein was shown to protect B-cells from apoptosis through inhibition of BAX transcription by activation of NFKB (P50/P65 heterodimer), which reduces the activity of the BAX promoter (Kontos, Fendri, Khabir, Mokdad-Gargouri, & Scorilas, 2013)

## Conclusion

Nasopharyngeal cancer is one type of cancer that has high morbidity and mortality rates throughout the world, including in Indonesia. One of the causes of this cancer is infection with the Epstein-Barr virus, but the initial diagnosis of nasopharyngeal cancer is difficult to establish because the early signs and symptoms of nasopharyngeal carcinoma are not specific. Examination of oncogene expression *latent membrane protein*-1 (LMP1)and gene expression *BCL2-associated X* (BAX) proved to be useful in the identification of nasopharyngeal cancer. LMP1 oncogene is an oncogene that functions as a tumor necrosis factor receptor (TNFR), so that apoptosis does not occur and promotes invasion and metastasis. The BAX gene is a

pro-apoptotic gene that inhibits cancer development, but its regulation will be downregulated by LMP1 because LMP can protect B-cells from apoptosis.

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