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Original Article

Correlation Between Immunohistochemical Expression of Epstein-Barr Virus Latent Membrane Protein-1 (LMP-1) and Clinical Stages of Sinonasal Carcinoma

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ABSTRACT

Introduction: *Sinonasal tumors are rarely found. The protected nature of the sinuses makes early detection of tumors growing in these areas difficult. The Epstein-Barr virus is one of the most common viruses found in humans; which expresses Latent Membrane Protein-1 (LMP-1) that can transform cell lines and alter cell phenotype due to its oncogenic potential.* ***Objective:*** *To investigate the relationship between immunohistochemical expression of Latent Membrane Protein-1 (LMP-1) from Epstein-Barr virus and clinical stages of sinonasal carcinoma.* ***Methods:*** *This cross-sectional study was carried out in DR. Wahidin Sudirohusodo General Hospital and anatomic pathology of Hasanuddin University Hospital, Makassar, South Sulawesi, Indonesia, from October 2016 to March 2017. Histopathological samples from biopsy specimen of all subjects diagnosed with sinonasal carcinoma in Otorhinolaryngology-Head and Neck Surgery outpatient clinic were taken and examined for LMP-1 expression using immunohistochemical staining. The samples were taken from paraffin block from patients.* ***Result:*** *There were 33 males (67.3%) and 16 females (32.7%) with sinonasal carcinoma who participated in this study. The majority of participants aged ≥50 years (25 people, 51.0%) and worked as farmers (27 people,*

55.1%). A significant correlation between LMP-1 expression and sinonasal carcinoma clinical stages was found (Spearman's correlation r = 0.355 and p <0.001). ***Conclusion:*** *Higher LMP-1 expression correlates with higher sinonasal carcinoma clinical stage in patients aged <50 years.*

1. INTRODUCTION

Sinonasal tumors, both benign and malignant, are rarely found. The frequency of malignant type is only 1% of all malignancies throughout the body and 3% of all malignancies in the head and neck. The nose and paranasal sinuses, also called sinonasal, are cavities bounded by the facial bones, making them 'protected' sites.[1] It makes early detection of tumors growing in these areas difficult. Thus, most patients who seek for medical care are already in advanced condition where the tumor has filled the nose and sinuses. Most of these tumors develop from the maxillary sinus and the most common histologic type is squamous cell carcinoma.[2-4]

The incidence of sinonasal malignant tumors is low in most of the population (<1.5/100,000 in men and < 0.1/100,000 in women). The highest incidence of sinonasal malignancies is found in Japan with 2-3.6/100,000 population per year. Case numbers are also high in certain areas in China and India.[2] In Makassar, South Sulawesi, Indonesia, the incidence of sinonasal carcinoma ranks second after nasopharyngeal carcinoma.[5]

The Epstein-Barr virus is one of the most common viruses found in humans.[6] Children do not experience any symptoms upon infection but Epstein-Barr virus infection in adults often lead to various clinical manifestations.[6] After a person is infected with the Epstein-Barr virus, the virus will be latent in the body.[6, 7] In latent infection, Epstein-Barr virus expresses Latent Membrane Protein-1 (LMP-1) which can transform cell lines and alter cell phenotype due to its oncogenic potential. LMP-1 is a viral oncogene that is similar to a cell surface receptor that can prevent Epstein-Barr virus-infected cells from apoptosis by inducing anti-apoptotic proteins. LMP-1 is also involved in signaling pathways that regulate cell proliferation and apoptosis by triggering cell progression and proliferation through the cell cycle (G1/S phase) and inhibition of apoptosis.[8, 9]

LMP-1 can induce various morphological arrangements and phenotypic effects on cells, one way is by inducing BCL-2 expression which results in a decrease in apoptotic indices that causes apoptosis inhibition and cell proliferation.[9] Positive LMP-1 has the potential to cause metastasis, accelerate disease progression, and suppress immune response. Some studies have shown that tumors with positive LMP-1 grow faster compared to negative LMP-1.[10]

Previous research has reported that in order to detect and localize the Epstein-Barr virus, EBER in situ hybridization can be considered as a gold standard.[11] Almost all of carcinomas associated with Epstein-Barr virus express EBER. EBER is an EBV-encoded small RNA 1 and 2 which is widely expressed in all forms of latent Epstein-Barr virus.[7] However, in Indonesia, EBER examination has some limitations such as the unstable nature of RNA which makes it less useful in tropical countries.[12]

The LMP-1 examination can detect the presence of a latent Epstein-Barr virus infection in the nasopharyngeal carcinoma tissue as LMP-1 expression is one of the prognostic factors of nasopharyngeal carcinoma.[13] Compared to EBER in situ hybridization, LMP-1 is also almost equally effective in detecting Epstein-Barr virus.[14, 15] Sinonasal lymphoepithelial carcinoma (SNLEC) or sinonasal undifferentiated carcinoma developing in areas with high incidence of nasopharyngeal carcinoma is not only morphologically identical to nasopharyngeal carcinoma but also consistently correlated with Epstein-Barr virus infection.[7, 16] This study aimed to determine the correlation between immunohistochemical expression of LMP-1 and clinical stage of sinonasal carcinoma. Such study has not been done in South Sulawesi.

2. METHODS

Study Design, Time, and Location

This cross-sectional study was carried out in DR. Wahidin Sudirohusodo General Hospital and anatomic pathology laboratory of Hasanuddin University Hospital, Makassar, South Sulawesi, Indonesia, from October 2016 until March 2017.

Population and Sample

Demographic data (age, gender, and occupation) and histopathological preparations from subjects with sinonasal carcinoma were included. Samples were grouped based on clinical stadium (stage I, stage II, stage III, stage IVA, stage IVB, stage IVC) and immunohistochemical staining assessing for LMP-1 expression. Clinical stadium was classified based on American Joint Committee on Cancer (AJCC) 2010 criteria. The LMP-1 expression was scored from 0 (no protein expression), +1 (1-25% protein expression on ¼ field of view [FOV]), +2 (26-50% protein expression on ¼-½ FOV), +3 (51-75% protein expression on ½- ¾ FOV), and +4 (>75% protein expression on 3/4 to whole FOV).

Data Analysis

Demographic data was presented using tables. The correlation between LMP-1 expression and clinical stage was assessed using Spearman's correlation test. A *p*-value of <0.05 was considered significant. All analysis was conducted using SPSS version 27.0 (SPSS Inc. Chicago, IL, USA).

3. RESULTS

From a total of 49 participants, 33 were males (67.3%) and 16 were females (32.7%). More subjects (25 subjects, 51.0%) were ≥ 50 years. Farmer was the most common occupation of this study (27 people, 55.1%) followed by housewife (15 subjects, 30.6%). The most common chief complaint was nasal obstruction (37 people, 75.5%). Other complaints were epistaxis (12.2%) and eye protrusion (12.2%). Based on histopathologic finding, squamous cell carcinoma was the most frequent type (35 samples, 71.4%) while the least common type was adenocarcinoma (3 samples, 6.1%) (Table 1).

Table 1. Sample Characteristics

Characteristics		Distribution	
		n	%
Sex	Male	33	67.3
	Female	16	32.7
Age (year)	15 – 49	24	49.0
	50 - 74	25	51.0
Anatomic pathology	Adenocarcinoma	3	6.1
	Adenoid cystic carcinoma	4	8.2
	Squamous cell carcinoma	35	71.4
	Undifferentiated Carcinoma	7	14.3
LMP-1 Expression	0	9	18.4
	+1	9	18.4
	+2	8	16.3
	+3	7	14.3
	+4	16	32.7
Clinical Stadium	I	1	2.0
	II	1	2.0
	III	19	38.8
	IVA	15	30.6
	IVB	10	20.4
	IVC	3	6.1
Occupation	Housewife	15	30.6
	Farmer	27	55.1
	Employee	4	8.2
	Others	3	6.0
Symptoms	Epistaxis	6	12.2
	Prominent eyes	6	12.2
	Nasal obstruction	37	75.5

The highest LMP-1 expression (+4) was found in 16 samples (32.7%) with varying clinical stages: stage III (4 samples, 8.2%), stage IVA (5 samples, 10.2%), stage IVB (6 samples, 12.2%) and stage IVC (1 sample, 2.0%). There were 9 samples from various clinical stages with no LMP-1 expression (Table 2).

Table 2. LMP-1 expression based on clinical stage of sinonasal carcinoma

LMP-1 Expression		Clinical stage						Total
		I	II	III	IVA	IVB	IVC	
0	n	1	0	5	3	0	0	9
	(%)	(2.0)	(0.0)	(10.2)	(6.1)	(0.0)	(0.0)	(18.4)
+1	n	0	0	5	1	2	1	9
	(%)	(0.0)	(0.0)	(10.2)	(2.0)	(4.1)	(2.0)	(18.4)
+2	n	0	0	4	3	1	0	8
	(%)	(0.0)	(0.0)	(8.2)	(6.1)	(2.0)	(0.0)	(16.3)
+3	n	0	1	1	3	1	1	7
	(%)	(0.0)	(2.0)	(2.0)	(6.1)	(2.0)	(2.0)	(14.3)
+4	n	0	0	4	5	6	1	16
	(%)	(0.0)	(0.0)	(8.2)	(10.2)	(12.2)	(2.0)	(32.7)
Total	n	1	1	19	15	10	3	49
	%	(2.0)	(2.0)	(38.8)	(30.6)	(20.4)	(6.1)	(100.0)
Result of Spearman correlation test		r=0.355				**<0.001**		

This study found a correlation between LMP-1 expression (Figure 1) and clinical stage with a correlation coefficient of r = 0.355 (p <0.001). This correlation can be age-bound; at age <50 there was a correlation of r = 0.547 (p = 0.003), but not in those aged ≥50 years (Table 3). Gender did not affect the correlation between LMP-1 expression and clinical staging (Table 4).

Figure 1. Immunohistochemical result. Negative (left) and positive (right) LMP-1 expression

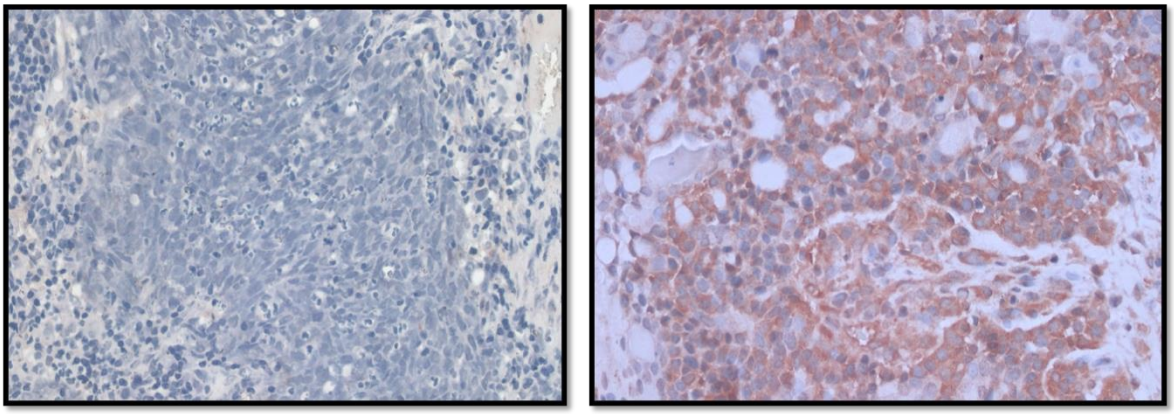

Table 3. Correlation of LMP-1 expression and clinical stage based on age

Stratification		Correlation of LMP-1 with clinical stage	
		Correlation coefficient	P-value
Age (year)	<50 y.o (n=24)	*r*=0.547	**0.003**
	≥50 y.o (n=25)	*r*=0.142	0.249

Table 4. Correlation of LMP-1 expression and clinical stage based on Sex

Stratification		Correlation of LMP-1 with clinical stage	
		Correlation coefficient	P-value
Sex	Male (n=33)	*r*=0.315	0.037
	Female (n=16)	*r*=0.372	0.078

No significant difference in LMP-1 expression based on tumor histopathologic outcome means that its expression level was not related to tumor histopathologic outcome (Table 5).

Table 5. Association between LMP-1 expression and tumor histopathology type

LMP-1 Expression	Tumor Histopathology Type N (%)				Total
	Adenocarcinoma	Adenoid Cystic Carcinoma	Squamous Cell Carcinoma	Undifferentiated Carcinoma	
0	0 (0)	1 (25)	7 (20)	1 (14.3)	9 (18.4)
1	1 (33.3)	0 (0)	8 (22.9)	0 (0.0)	9 (18.4)
2	1 (33.3)	0 (0.0)	6 (17.1)	1 (14.3)	8 (16.3)
3	0(0.0)	1 (25.0)	4 (11.4)	2 (28.6)	7 (14.3)
4	1 (33.3)	2 (50.0)	10 (28.6)	3 (42.9)	16 (32.7)
Total	3 (100.0)	4 (100.0)	35 (100.0)	7 (100.0)	49 (100.0)

Kruskal wallis test p= 0,584

4. DISCUSSIONS

This study showed that there was a significant correlation between LMP-1 expression and clinical stages of sinonasal carcinoma (Spearman correlation test r = 0.355 and p <0.001) where a higher level of LMP-1 expression is associated with a higher clinical stage.

The higher incidence of sinonasal carcinoma in males are probably correlated with occupational environmental factors in men potentially leading to cancer; however, the scientific evidence for this is still rare. Most of literature found that the incidence of sinonasal carcinoma increases during the 6th decade of life with a male and female ratio of 2: 1.

The age characteristic showed the most frequent age group was ≥ 50 years. This was in accordance with previous research where the highest age group was in the 6th decade.[17] Another study also found that the highest incidence of sinonasal carcinoma was in middle-aged males (46-50 years).[18] Until now, there has been no scientific explanation for this finding. This may happen because of advanced age are more susceptible to cancer and other diseases caused by decreased body metabolism

resulting in weakening of the immune system, as well as unhealthy lifestyles since youth and environmental factors.

Our data found that farmers were the most common occupation followed by housewife. Farming is correlated with the use of formaldehyde which can be found in cigarettes and pesticides. This finding is supported by a study that reported most patients with sinonasal carcinoma were farmers (36.4%) who were frequently exposed to chemicals and industrial materials.[18] A study revealed that formaldehyde was classified as carcinogenic to humans, although with limited evidence on the incidence of sinonasal carcinoma.[19]

This study also showed that nasal obstruction was the most common presenting sign. Data from previous study showed that the most common symptoms were nasal obstruction, proptosis, cranial nerve palsy, periorbital swelling, diplopia, epistaxis, and periorbital pain.[18] This occurs because the enlarged tumor involves several paranasal sinuses and causes damage to the sinus and surrounding walls. On the other hand, Poursadegh *et al* found a different result where facial swelling was the main presenting complaint; however, they also found that Squamous cell carcinoma was also the most common histopathologic finding which was consistent with our result.[20]

This study found that LMP-1 expression was not only found in severe clinical stage but also in early stages. This occurred because the researcher did not conduct serologic examination of Epstein-Barr virus prior to LMP-1 examination which was one of the limitations of this study.

A weak correlation between LMP-1 expression and clinical stages of sinonasal carcinoma patients in Makassar. Similarly, another study found that LMP-1 protein expression had significant correlation with clinical stage of sinonasal carcinoma.[21] These results suggested that EBV infection is indeed one of the risk factors in sinonasal carcinoma. The correlation in our study may be affected by age. Patients <50 years old had a strong significant correlation to influence the correlation between LMP-1 and clinical stage. However, no correlation was found in those aged ≥50 years. Gender did not affect the correlation between LMP-1 and clinical stage.

The expression of LMP-1 affects the stage of sinonasal carcinoma. This may be due to the role of LMP-1 as carcinogenic, antiapoptosis and a high tendency to tumor metastasis.[22-24] However, in nasopharyngeal carcinoma, there was no significant correlation between nasopharyngeal carcinoma stage and LMP-1 expression ($p>0.05$).[25] There is no scientific explanation for the correlation between age and LMP-1 expression in sinonasal carcinoma.

The limitation of this study was we were unsure whether the tumor came solely from sinonasal or derived from nasopharynx that extended to the sinonasal. This study also could not determine the type of tumor histopathology that affects LMP-1 expression because the limited number of samples.

5. CONCLUSION

There was a significant correlation between LMP-1 and sinonasal carcinoma clinical stage, where higher LMP-1 expression corresponded to more severe clinical stadium in subjects <50 years.

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Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.