

RESEARCH COMMUNICATION

LMP1 and LMP2 may be Prognostic Factors for Outcome of Therapy in Nasopharyngeal Cancers in Indonesia

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Abstract

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy that is invasive and metastasizes easily. In several Asian countries it is the most commonly found of the head and neck malignancies. Epstein Barr virus (EBV) infection is one of the agents causing NPC, so that expression of LMP1 and LMP2 may affect the outcome of therapy, metastasis, recurrence, and survival of NPC patients. This study aimed to investigate their expression in relation to therapy outcome and survival in a series of Indonesian NPC patients. The methods used were nested case control and Kaplan-Meier survival analysis. Differences in therapy outcome in relation to LMP1 and LMP2 expression were analyzed through chi square statistics. As a result, in post treatment NPC, there was a significant difference in therapy outcome between LMP2 (+) compared to LMP2 (-) ($P = 0.001$). There was also a significant difference in 24-months-survival between NPCs expressing LMP1 (+) or LMP2 (+) compared to those expressing LMP1 (-) or LMP2 (-).

Keywords: NPC - LMP1 - LMP2 - prognosis factors - Indonesia

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy which has quite high frequency in Asian countries, especially in males (Pathmanan, 1997). It has different characteristics with a tendency to be invasive and metastasize easily compared to other head and neck malignancies (Horikawa et al., 2000; Ma et al., 2007; Tang et al., 2008).

NPC is rarely found in Europe and America, but in Asian especially southern China it has an incidence rate of 40-50/100.000 population per year. Unspecific early symptoms cause NPC patients to present with advanced stages, resulting in unsatisfactory therapy outcome.

Epstein Barr Virus (EBV) infection is one of the risk factors for NPC and several other malignancies. EBV is a pathogenic virus infecting almost 90% of the world's population which can be latent and persist for a lifetime (Edward et al., 2004; Allen et al., 2005). EBV infection in the body may become lytic in which active virus is contagious and may become latent or persistent, inducing proliferation of infected cell and may progress into NPC, malignant lymphoma, Burkitt lymphoma, oral hairy leucoplakia etc (Brooks, 1995; Middeldorp, 2003).

In NPC, EBV infection become latent and mostly found in WHO type II NPC (non keratinizing carcinoma) and WHO type III NPC (undifferentiated carcinoma), and in WHO type III EBV infection is found in nearly 100% cases (Pegtel et al., 2005). EBV expresses several

proteins including LMP1 and LMP2. LMP1 may activate nuclear factor kappa beta ($\text{NF}\kappa\beta$) which plays a role in malignancy, causing tumor to be resistant to antitumor agents, resulting in failure of therapy (Li et al., 2003; Uzo et al., 2004; Wakizaka et al., 2005; Yoshizaki et al., 2005). LMP2 is a hydrophobic membrane protein, that has two forms: LMP2a and LMP2b. LMP2a may play a role in disturbing signal transduction of B cell, causing EBV infection to become latent (Longneker, 2000 cit; Zetterberg, 2005). Other function of LMP2 is: 1) inhibit apoptosis by blocking the function of Bad, 2) promote metastasis by degrading β Catenin (Yoshizaki et al., 2005), while the function of LMP2b remains unclear (Khanna, 1995).

Materials and Methods

This study aimed to investigate whether there is any difference in outcome of therapy and 24-months survival between NPC patients given standard therapy in Sardjito Hospital expressing LMP1 and LMP2. Methods used were case control and Kaplan Meier survival analysis. LMP1 and LMP2 expressions were examined by immunohistochemistry before NPC patients received any therapy. Outcome of therapy including response (+)/adequate result (control) and response (-)/inadequate result (case) was defined by post therapy biopsy as a gold standard performed 8-12 weeks after complete treatments. Difference of therapy results was analyzed through chi

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square.

NPC patients satisfying inclusion and exclusion criteria was enrolled in ENT Department of Sardjito Hospital consecutively (Consecutive sampling) until the required number of samples is fulfilled.

The expressions of LMP1 and LMP2 were performed through immunohistochemistry by an Anatomic pathologist, LMP1 expression was examined with monoclonal antibody CS1-4 produced by Novocastra, while LMP2 expression was examined with monoclonal antibody SC 16459 produced by Santacruz, containing monoclonal antibody LMP2a and LMP2b. The expression of LMP21 and LMP2 was quantified by the sum of expressed cell score expressed cell color intensity (Khabir et al., 2005).

Results and Discussion

Fifty six NPC patients in stage III and IV with no distant metastasis satisfying inclusion and exclusion criteria consisted of 28 patients obtaining response (+)/adequate response and 28 patients obtaining response (-)/inadequate response. Characteristic and homogeneity of subjects are presented on Table 1.

Since 2004, Sardjito Hospital has applied the golden

Table 1. Characteristic of Subjects

	Response of therapy(-)	Response of therapy(+)	P
Age			
11-20	2	1	0.93
21-30	2	2	
31-40	3	2	
41-50	11	11	
51-60	6	5	
61-70	4	5	
> 70	1	1	
Sex			
Male	19	21	0.55
Female	9	7	
Stage			
III	14	16	0.55
IVA	3	3	
IVB	11	9	
PA			
WHO II	2	-	0.30
WHO III	26	28	
Therapi			
Protocol A	12	8	0.26
Protocol B	26	20	

standard for stage III and IV chemotherapy including therapy protocol A which is a neoadjuvant chemotherapy continued with radiotherapy, and therapy protocol B which is a neoadjuvant chemotherapy continued with radiotherapy and brachytherapy.

In post treated NPC with therapy response (-), the mean of LMP1 found was 9.1429 with SD 1.8372, and the mean of LMP2 was 4.90 SD 1.919, while in NPC with therapy response (+) the mean of LMP1 was 5.307 SD 0.96 while mean of LMP2 was 1.40 SD 0.57. T-test analysis was performed and there was a significant difference in the expressions of LMP1 and LMP2 between therapy outcomes (P=0.001) (Table 2).

The significant difference between the mean of LMP1 and LMP2 in relation to therapy outcome has proven that LMP1 and LMP2 play a major role in outcome of therapy, this is in accordance with a theory proposing LMP1 as an antiapoptotic agent and affecting tumor resistance against antitumor drugs (Li et al., 2003; Uzo et al., 2004; Wakizaka et al., 2005; Yoshizaki et al., 2005), while LMP2a may play a role in disturbance of B cell signal transduction, enabling latent EBV infection (Longneker, 2000; Zetterberg, 2005), and inhibit apoptosis which decrease therapy outcome (Yoshizaki et al., 2005).

To calculate odds ratio (OR) the quantitative data of expressions of LMP1 and LMP2 was converted by Receiver Operating Curve (ROC). Lowest LMP1 expression was 3.0 and the highest was 11.6. Cutoff point to define the difference between expression LMP1 (+) and LMP1 (-) was 7.20, while lowest LMP2 expression was 0 and the highest was 7.8, and the cutoff point to define the difference between LMP2(+) and LMP2(-) was 2.70.

Results of bivariate analysis of expressions of LMP1 and LMP2 and other variables showed a significant difference in outcome of therapy between LMP1>7.2 compared to LMP1<7.2 (P=0.001; OR 27.6) and significant difference in outcome of therapy between LMP2>2.7 compared to LMP2<2.7 (P<0.001; OR 50.0). There was no significant difference in outcome of therapy between different sex, stages, and anatomy pathology results (Table 3).

Bivariate analysis of the expressions of LMP1 and LMP2 and other variables toward the 24-month survival rate showed significant difference between LMP1>7.2 and LMP1<7.2 (P=0.003; OR=9.28) and significant difference between LMP2>2.7 and LMP2<7.2 (P=0.018; OR=5.10) (Table 4). These results support the analysis that expression of LMP1 and LMP2 cause a decrease in the success of therapy which further decrease survival.

These results also support the theory which stated that one role of LMP1 in NPC is to promote metastases by activating COX-2 enzyme responsible in angiogenesis process (Muroso et al., 2001). An increase in metastases further causes a decrease in survival.

Table 2. Difference in Mean Protein Expression between Therapy Outcome

Protein	Therapy outcome (-)			Therapy outcome(+)			P
	Mean	Sd	N	Mean	Sd	N	
LMP 1	9.1429	1.83720	28	5.5307	0.964760	28	0.001*
LMP2	4.9000	1.91949	28	1.4000	0.57803	28	0.001*

Table 3. Bivariate Analysis of Protein Expression and other Variables Toward the Outcome of Therapy

Variables		Therapy outcome		OR (95 % IK)	P
		Negative N (%)	Positive N (%)		
Sex	Male	19 (67,9%)	20 (71,4%)	0,844 (0,270 - 2,642)	0,771
	Female	9 (32,1%)	8 (28,6%)		
Stage	IV B	11 (39,3%)	9 (32,1%)	1,4 (0,39 - 5,09)	0,846
	IV A	3 (10,7%)	3 (10,7%)		
	III	14 (50,0%)	16 (57,1%)		
Pathologic	WHO III	27 (96,4%)	28 (100,0%)	1	0,313
	WHO II	1 (3,6%)	-		
LMP 1	≥ 7,2	23 (82,1%)	4 (14,3%)	27,60 (6,58 - 115,77)	<0,001*
	< 7,2	5 (17,9%)	24 (85,7%)		
LMP 2	≥ 2,7	24 (85,7%)	3 (10,7%)	50,0 (10,11 - 247,23)	<0,001*
	< 2,7	4 (14,3%)	25 (89,3%)		

Table 4. Bivariate Analysis of Protein Expression Toward 24-month Survival Rate

Variable		Outcome		OR (95 % IK)	P
		Death n (%)	Survive n (%)		
Sex	Male	11 (84,6%)	28 (65,1%)	2,95 (0,58 - 15,07)	0,180
	Female	2 (15,4%)	15 (34,9%)		
Pathologic	WHO III	13 (100,0%)	42 (97,7%)	0,65 (0,06 - 7,74)	0,579
	WHO II	0	1 (2,3%)		
LMP 1	≥ 7,2	11 (84,6%)	16 (37,2%)	9,28 (1,82 - 47,30)	0,003*
	< 7,2	2 (15,4%)	27 (62,8%)		
LMP 2	≥ 2,7	10 (76,9%)	17 (39,5%)	5,10 (1,22 - 21,25)	0,018*
	< 2,7	3 (23,1%)	26 (60,5%)		

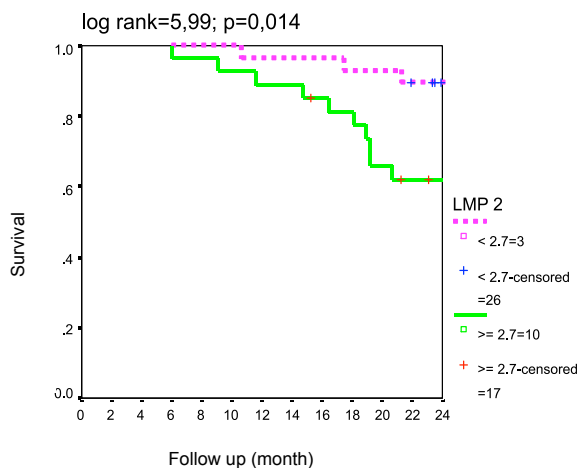


Figure 1. Difference in 24-month Survival between LMP2 > 2.7 and LMP2 < 2.7

By observing 24 months post therapy, using Kaplan Meier survival analysis, we found significant difference in 24-month survival between NPC expressing LMP1>7.2 and LMP1<7.2 (P=0.002; log rank 9.79), which can be interpreted that NPC patients with the expression of LMP1<7.2 have the chance to survive 9.79 times higher than those with the expression of LMP1>7.2.

From the analysis of LMP2 expression and 24-month survival using Kaplan Meier survival analysis, we found significant difference between NPC expressing LMP2>2.7 and LMP2<2.7 (P=0.014; log rank 5.99) (Figure 1). It can be interpreted that NPC patients with the expression of

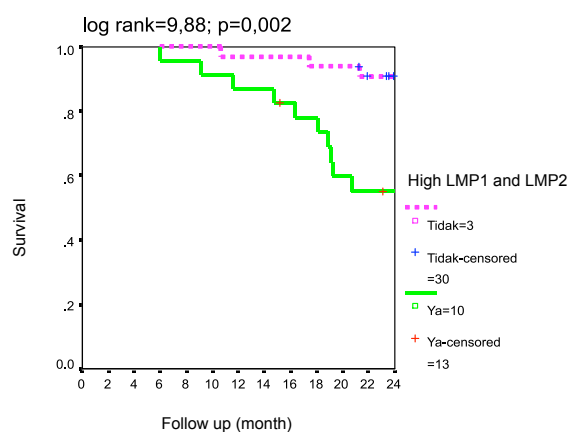


Figure 2. Difference in 24-month Survival between NPC Expressing LMP1>7.2 and LMP2>2.7 Compared to those Expressing LMP1<7.2 and LMP2<2.7

LMP2<2.7 have the chance to survive 5.99 times higher than those with the expression of LMP2>2.7.

From the analysis of 24-month survival rate difference between NPC expressing LMP1>7.2 and LMP>2.7 compared to NPC expressing LMP1 >7.2 and LMP2 >2.7, we found significant difference of 24-month survival between NPC expressing LMP1>7.2 and LMP>2.7 compared to NPC expressing LMP1>7.2 and LMP2>2.7 (P=0.002; log rank 9.88) (Graph 3). From this result, it can be assumed that NPC patients with the expression of LMP1<7.2 and LMP2<2.7 have the chance to survive 9.88 higher than those with the expression of LMP1>7.2

and LMP2>2.7.

In conclusion, in NPC expressing either LMP1 >7.2 or LMP2 >2.7 and both, there were failures in the outcome of therapy and 24-month survival compared to NPC which expressed either LMP1 <7.2 or LMP2 <2.7. Further studies need to be carried out by extending the duration of observation into 5 years and by analyzing other proteins.

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