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Effect of LMP-1 and mutant p53 on the Prognosis of Undifferentiated Type of Nasopharyngeal Carcinoma

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Abstract: Undifferentiated carcinoma is a subtype of NPC with a higher EBV antibody titer than healthy people. The carcinogenic effect of EBV involves LMP-1 associated with poor prognosis, and mutant p53 predicts tumor recurrence. This study aims to assess the effect of LMP-1 and mutant p53 on the prognosis of NPC patients. 40 paraffin blocks were stained with LMP-1 and p53 mutant. LMP-1 was positive when the cytoplasm and tumor cell membranes were brown, and mutant p53 was positive for the brown tumor cell nucleus. Pearson correlation test was performed. Most respondents were in the age group > 40 years, males, regional lymph node involvement (N) in group N1, and no metastasis (M). The expression levels of LMP-1 and mutant p53 were strongly positive. The study showed the correlation between LMP-1 with age (p 0.327), gender (p 0.599), category N (p 0.512), category M (p 0.019) and the correlation between mutant p53 with age (p 0.329), gender (p 0.981), category N (p 0.013), category M (p 0.705). LMP-1 expression with mutant p53 (p 0.760). It can be concluded that LMP-1 and mutant p53 could be used as prognostic factors in NPC patients.

Keywords: LMP-1; Nasopharyngeal Carcinoma; p53 Mutant; Prognosis

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is the 5th leading cause of death in Indonesia and 8th in the world. In 2020, there were 19,943 new cases (5.0%) in Indonesia, with a death rate of 13,399 cases (5.7%). The incidence and death rates caused by NPC are not categorized as large, but NPC remains a concern because men with 10.7/100,000 cases dominate it compared to women with 3.00/100,000 cases. The ratio between men and women is 3-4:1. The incidence rate is 6.8/100,000 cases, and the death rate is 4.7/100,000 cases.[1]

NPC is divided into three subtypes, namely, NKSCC/NK-KNF (undifferentiated subtype and differentiated subtype), KSCC (undifferentiated and differentiated), and basaloid squamous cell carcinoma (BSCC). The second subtype is divided into differentiated and undifferentiated carcinomas.[2-3] NPC's risk factors include adult males, 30-50 years old, family history of NPC, consumption of preserved foods, salted fish, smoking, and EBV infection.[4] Undifferentiated carcinoma is the most common histological subtype of NPC found in Southeast Asia and Indonesia. NPC patients from various countries ranged from 4 to 91 years, with a peak incidence at 50 to 60 years in the Chinese population.[5]

According to the Global cancer statistics from the International Agency for Research on Cancer, there were more than 84,000 new NPC cases in 2008, with 80% of cases in Asia and 5% in Europe. NPC is characterized by poor or undifferentiated carcinoma. NPC is endemic in South China and Southeast Asia, with an annual incidence of 15-50 cases per 100,000. Medium incidence rates of NPC are seen in Southeast Asia, including Singapore (15/100,000), Malaysia (9.7/100,000), Vietnam (7.5/100,000), Taiwan (7/100,000), and the Philippines (6.4/100,000).[6] In Indonesia, with a population of 225 million people, NPC poses socio-economic problems, with an estimated overall incidence of 6.2/100,000 or about 12,000 new cases per year. This disease is 100% associated with Epstein Barr virus (EBV) infection, especially the most common type of undifferentiated carcinoma WHO type III.[5]

Non-keratinizing squamous cell carcinoma and undifferentiated carcinoma have a higher propensity to metastasize than keratinizing squamous cell carcinoma. On the other hand, non-keratinizing squamous

cell carcinoma and undifferentiated carcinoma have a higher degree of radiosensitivity, so they have a better prognosis.[7] The treatment results in 3 years disease-free and overall survival of approximately 70% and 80%, respectively.[5]

EBV has a carcinogenic effect through mechanisms that include latent membrane protein (LMP) consisting of LMP-1 and LMP-2 as well as microRNA. LMP-1 is an integral protein encoded by EBV in the latent phase and is associated with poor prognosis of NPC as LMP-1 is the most influential on tumor cells growth processes such as migration, proliferation, metabolism, and tumorigenesis.[6]

LMP1 is expressed only on neoplastic (tumor) cells. LMP1 is expressed in most patients with pre-malignant lesions on the surface of their tumor tissues.[8] It is the main oncogene in NPC tumorigenesis so that the expression of EBV LMP1 mRNA acts as a biological marker of latent EBV infection.[9]

The gene most frequently altered in human tumors is TP53 encoding the p53 protein. TP53 mutations are associated with a poor prognosis in many sporadic cancers. The primary outcome of TP53 mutations is loss of function of wild-type p53, which represents an advantage during cancer progression by eliminating intrinsic tumor suppressor's response cells, such as aging and apoptosis. Genes that make proteins are found in the nucleus of cells and play a key role in controlling cell division and cell death. Mutations (changes) in the p53 gene can cause cancer cells to grow and spread in the body.[10]

The p53 gene has been mapped to chromosome 17. Inside cells, the p53 protein binds to DNA, which stimulates other genes to produce a protein called p21 that interacts with the cell division-stimulating protein (cdk2).[11]

An association between EBV infection and p53 expression was reported in idiopathic pulmonary fibrosis, gastric adenoma, gastric carcinoma, head and neck non-Hodgkin's lymphoma (NHL), Nasopharyngeal Cancer, Burkitt's Lymphoma, and Gastric Carcinoma. In addition, p53 concentrations were reported to determine cell cycle arrest and apoptosis in EBV-infected B cells.[12] A positive mutant p53 expression will provide a better prognosis for the outcome of radiation therapy in NPC. NPC's prognosis with a positive mutant p53 expression will increase compared to a negative p53 mutant.[13] In head and neck cancer, high mutant p53 mutation rates have been associated with tobacco consumption and poorer prognosis.[14]

When mutant p53 is lost or mutant p53 is mutated, it will play a role in tumor development, progression, and chemotherapy resistance. Loss of mutant p53 also decreases apoptosis and sensitivity to radiotherapy or chemotherapy.[15] Immunohistochemical studies showed that EBV infection in NPC was associated with the accumulation of mutant p53 protein, not with BCL-2 protein. However, other studies have concluded that EBV is an important etiologic factor that may involve overexpression of mutant p53 and BCL-2.[16]

Overexpression of mutant p53 protein has a close relationship with the increased incidence of primary tumors and can be used as a marker for the molecular stage of head and neck malignant tumors to predict tumor recurrence and tumor response to neoadjuvant chemotherapy in head and neck malignant tumors.[17] There are few studies on LMP-1 and mutant p53 in NPC available, and reports on NPC patients are inconsistent. For example, in the study of Tabyaoui et al., there was no significant correlation between the expression of LMP-1 and mutant p53 with histological type, age, and gender distribution shown in NPC.[18]

According to histologic subtype, another study explained that LMP-1 was detected in 35 NPC-positive cases, in 55% undifferentiated squamous cell carcinoma, 28% keratinized squamous cell carcinomas, and 21% in non-keratinized squamous cell carcinoma.[19] Another study found that NPC cells had increased mutant p53 levels, with high LMP-1 levels correlated with higher mutant p53 expression. LMP-1 can cooperate with mutant p53 to induce the growth of NPC cells.[20] Based on the background, the study aims to identify the effect of LMP-1 and mutant p53 on the prognosis of Nasopharyngeal Carcinoma Patient Undifferentiated Type.

MATERIALS AND METHOD

This study is an observational analytic study with a cross-sectional approach aiming to assess the immunohistochemical expression of LMP-1 and mutant p53 for the prognosis of NPC patients. This research was conducted at the Anatomical Pathology Laboratory, Dr. Kariadi Semarang.

The target population in this study were Hematoxillin Eosin (HE) slides and tissue paraffin blocks originating from the nasopharynx with NPC diagnosis. The research sample included the target population that met the inclusion and exclusion criteria. Researchers took a sample of 40 samples. The independent variables in this study were the expression of LMP1 and P53. Prognosis consisted of age, gender, regional lymph node involvement (N), and metastases (M) in NPC patients.

LMP-1 immunoexpression was declared positive when the cytoplasm and tumor cell membranes were brown. The calculation of tumor cells with LMP-1 immunoexpression was viewed using a microscope with an objective lens magnification of 40x by random sampling; 10 fields of view were taken. Based on distribution, 0 if no cells are positive, 1 if positive cells are > 0-1%, 2 if positive cells are > 1-10%, 3 if positive cells are > 10-33%, 4 if the cells are positive > 33-66%, and 5.If the cells are positive > 66%. By intensity: 19 0 if no color. 1 if color is present and intensity is weak (light brown/pale). 2 if color is present and medium intensity (brown). 3 if there is color and strong intensity (dark brown). The final score is the increase between the distribution and the intensity, namely: 0-8, and the measuring scale is ordinal. 0: negative, 1-2: positive weak. 3-4: medium positive. 5-8: strong positive.

Immunoexpression of p53 was declared positive when the nucleus of the tumor cells was brown. The counting of tumor cells with p53 immunoexpression was viewed using a microscope with an objective lens magnification of 40x. Random sampling took 10 fields of view. Based on distribution, 0 if no cells are positive, 1 if positive cells are > 0-1%, 2 if positive cells are > 1-10%, 3 if positive cells are > 10-33%, 4 if the cells are positive > 33-66%, and 5 If the cells are positive > 66%. Based on intensity, 0 if color is none, 1 if color is present and intensity is weak (light brown/pale), 2 if color is present and medium intensity (brown), 3 if there is color and strong intensity (dark brown). The final score is the increase between the distribution and the intensity, namely: 0-8, and the measuring scale is ordinal.35 0: negative, 1-2: positive is weak, 3-4: medium positive. 5-8: strong positive. Data processing was carried out with the help of statistical software and Microsoft Excel 2019. The correlation between LMP-1 expression and p53 expression was tested using the Pearson Correlation test. P-value < 0.05 was considered statistically significant.

RESULTS

Characteristics of age, gender, lymph node involvement and metastases and their relationship to LMP-1 and mutant p53 in NPC patients can be seen in Table 1.

Tabel 1. Characteristics of Age, Gender, Lymph Node Involvement, Metastases, and Their Relationship to LMP-1 and Mutant P53 in NPC Patients

Clinical Parameters	**Total (%)**	**LMP-1**		**P *(Sig.(2-tailed))***	**Mutant p53**			**P *Sig.(2-tailed))***
		(+) Positive (%)	(-) Negative (%)		(+) weak (%)	(+) moderate (%)	(+) strong (%)	
Age				0.327				0.329
>46 years old	27(67.5%)	27(67.5%)	0		0	1(2.5%)	26(65%)	
≤46 years old	13(32.5%)	13(32.5%)	0		1(2.5%)	0	12(30%)	
Gender				0.599				0.981
Male	27(67.5%)	27(67.5%)	0		1(2.5%)	0	26(65%)	
Female	13(32.5%)	13(32.5%)	0		0	1(2.5%)	12(30%)	
Lymph nodes				0.512				0.013*
No	2(5%)	2(5%)	0		0	1(2.5%)	1(2.5%)	
N1	38(95%)	38(95%)	0		1(2.5%)	0	37(92.5%)	
Metastasis				0.019*				0.705
No	3(7.5%)	3(7.5%)	0		0	0	3(7.5%)	
Yes	37(92.5%)	37(92.5%)	0		1(2.5%)	1(2.5%)	35(87.5%)	

Characteristics of the age distribution of NPC patients in this study showed 27 (67.5%) NPC patients were > 46 years old, while 13 (32.5%) NPC patients were ≤ 46 years old. Based on these data, it was concluded that

most NPC patients were in the age group > 46 years. Characteristics of the gender distribution of NPC patients in this study showed that 27 (67.5%) were male, while 13 (32.5%) were female. Based on these data, it was concluded that most NPC patients were male. Meanwhile, characteristics of the distribution of lymph node involvement (N) in NPC patients in this study showed that 2 (5%) NPC patients were in the N0 category, while 38 (95%) NPC patients were in the N1 category. Based on these data, it was concluded that most NPC patients were included in category N1. The characteristics of the distribution of metastases in NPC patients in this study showed that 37 (92.5%) NPC patients did not have metastases, while 3 (7.5%) other NPC patients had metastases. Based on these data, it was concluded that most NPC patients did not have metastases. The level of expression of LMP-1 in NPC patients in this study showed that 40 (100%) NPC patients had positive LMP-1 expression.

The results showed that 1 (2.5%) NPC patient had a weakly positive mutant p53 expression level, 1 (2.5%) NPC patient had a moderate positive mutant p53 expression level and 38 (95 %) NPC patients had a strong positive mutant p53 expression level. Based on the results of this study, it was concluded that most NPC patients had a strong positive mutant p53 expression level.

The results of the correlation between the expression of LMP-1 and mutant p53 with lymph node involvement (N) and metastasis (M) revealed that: 1) Based on the significance value of Sig. (2 tailed) between LMP-1 expression and lymph node involvement (N), it was 0.512 (> 0.05), the calculated r-value was -0.107 < r table 0.312. It can be concluded that there was no relationship between LMP-1 expression and nodes lymph involvement (N). 2), Based on the significance value of Sig. (2 tailed) between LMP-1 expression and metastasis (M), it was 0.019 (<0.05), and the calculated r-value was – 0.370 > r table 0.312. As the calculated r-value in this analysis is negative, indicating that the correlation between LMP-1 expression and metastasis (M) is negative, it can be concluded that the higher the expression of LMP-1 is, the lower the incidence of metastasis (M) in NPC patients will be. 3) Based on the significance value of Sig. (2 tailed) between mutant p53 expression and lymph node involvement (N), it was 0.013 (< 0.05), r value 0.389 > r table 0.312. Since the r-count in this analysis is positive, indicating that the correlation between mutant p53 expression and lymph node involvement (N) is positive, it can be concluded that the higher the mutant p53 expression is, the more the lymph node involvement (N) in NPC patients will be. 4) Based on the significance value of Sig. (2 tailed) between mutant p53 expression and metastases (M), it was 0.705 (> 0.05), r value 0.062 < r table 0.312,. It can be concluded that there was no relationship between mutant p53 expression and metastasis (M). LMP-1 expression in nasopharyngeal tissue of nasopharyngeal carcinoma patients can be seen in Figures 1 and 2.

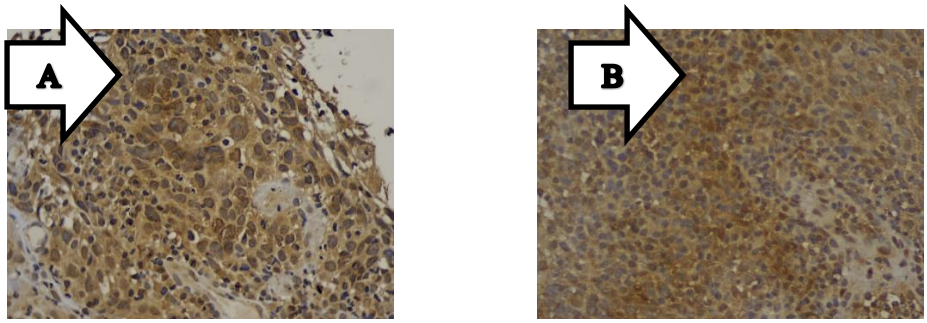
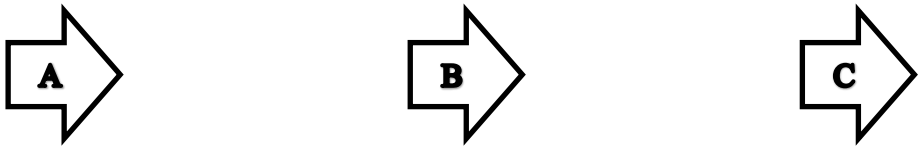


Figure 1. LMP-1 medium expression (A). LMP-1 strong expression (B).



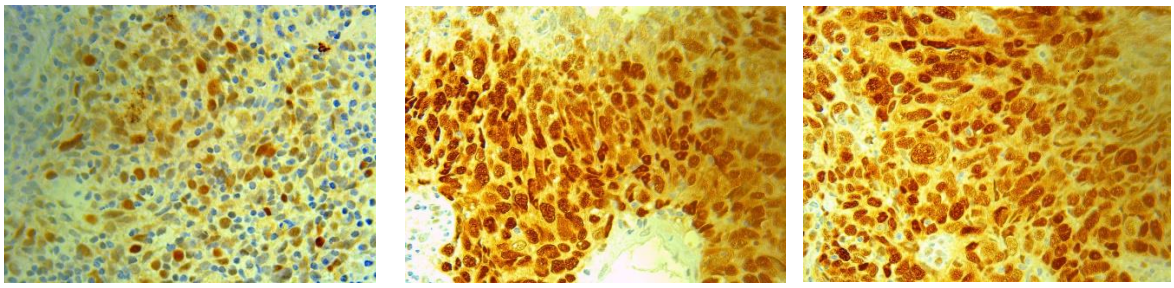


Figure 2. Mutant p53 week expression (A). Mutant p53 medium expression (B).
Mutant p53 strong expression (C).

DISCUSSION

Patient age distribution

The results of this study are in line with research conducted by Dawolo, which stated that most nasopharyngeal cancer patients were in the age group 46-55 years (30.91%).[22] The similar aspect was also explained by Barns that in areas with a high risk of nasopharyngeal cancer, the incidence increased after the age of 30 and peaked at the age of 40- 60 years.[23]

Furthermore, the results of this study are in accordance with the study conducted by Fatusi *et al*. explaining that NPC patients from various countries can suffer from ages from 4 to 91 years, with a peak incidence at 50 to 60 years in the Chinese population. Generally, NPC is rare in individuals under 20 years old (less than 1%), whereas a bimodal age distribution has been described in northern Africa, with 20% of patients under 30 years of age.[24]

Another study by Chang YL also showed similar results to our study where 323 new patients were treated between 1998 and 2004 at the National University Hospital in Singapore; 36% to 40% were diagnosed at the age of 41 to 50 years. According to the literature, the overall incidence peaks at 50 to 60 years of age. In high-risk areas such as Hong Kong, the incidence of NPC in each gender increases sharply from 20 years and above and peaks between 40 and 60 years.[25]

Another study by Barnes also explained that age is also a risk factor for nasopharyngeal cancer. The incidence of nasopharyngeal cancer increases after the age of 30 and peaks at the age of 40-60 years. After the age of 60, the incidence begins to decline. Meanwhile, another study by Tsao also revealed that the incidence of nasopharyngeal cancer commonly occurred at the age of 45 to 54 years.[26]

Guo *et al*. also stated that the incidence of nasopharyngeal cancer began to increase after the age of 30 years; 93% occurred after passing the age of 30 years, with the highest peak at the age of 45-55 years. The study results found a significant reduction in the incidence of nasopharyngeal cancer in the age group > 65 years. It is in line with research stating that the incidence began to decline after the age of 60.[27]

Kumar *et al*. explained that nasopharyngeal cancer is mostly found in productive ages, especially at the age of 46-55 years, as cancer growth takes a long time. The increasing incidence of cancer at this age can be explained by the accumulation of somatic mutations associated with cancer. Decreased immune system resistance due to age is a factor that may increase the risk of cancer.[28]

Gender distribution

The research by Ismail and Savitri at the Dr. Central General Hospital Wahidin Sudirohusodo and Hasanuddin University Hospital revealed that male NPC patients had a higher percentage than females. The Central General Hospital, Dr. Wahidin Sudirohusodo, found as many as 64.18% of male patients, while patients with female gender were 35.82%. Meanwhile, at Hasanuddin University Hospital, 55.56% of NPC patients were male, and 44.44% were female.[29] IARC states that men are more likely to experience NPC of 72.73%.[29] It is also stated that men had a higher risk of developing NPC than women, about 2-3 times higher. GLOBOCAN also states that men had 1.38 times higher risk of developing nasopharyngeal cancer than women.[30]

Xie *et al*. concluded that the incidence rate of NPC across populations was male predominance. In most populations, NPC's male to female incidence ratio is approximately 2-3:1.[31] Sulaksana *et al*. denoted that the number of male respondents was greater than female respondents with a ratio of 2.5:1. These results are

consistent with many previous studies reporting that NPC is more common in men than women, with a 2-3:1 ratio.[32] Xie *et al*. explained that the male predominance in NPC incidence could be partly explained by differences in the prevalence of several environmental risk factors, such as smoking and occupational exposure to hazardous. It is also possible that some intrinsic exposures, such as sex hormones, may explain the observed male predominance of the protective effects of endogenous estrogens.[31] Another study by Utama also explained that NPC is influenced by gender. It occurred since some of the most common factors that cause NPC are often found in men, such as smoking, drinking alcohol, and exposure to wood dust.[33]

Distribution of regional lymph node involvement (N)

The results of this study were different from other studies by Utama, which concluded that based on the description of cervical lymph node metastases, the most common result was lymph nodes in the N3 group of 7 patients (58.3%). Furthermore, the most N1 lymph nodes were 3 patients (25%), while N0 and N2 were 1 patient (8.33%).[33]

Licitra explained that the neck lymph nodes that are painless and grow slowly often make the patient ignore complaints. Therefore, patients carried out medical checks up when they were already in an advanced stage.[34]

Distribution of distant metastases (M)

This study differed from other studies by Zheng, who explained that the incidence of metastases was much higher in locally advanced NPC, and the most common sites were bone, lung, and liver.[35] According to Brennan, NPC usually originates from the lateral wall of the nasopharynx, which includes the fossa of Rosenmuller. They may extend into or out of the nasopharynx to other lateral walls and posterosuperior to the skull base or palate, nasal cavity, or oropharynx, then usually metastasizes to the glands neck lymph. Distant metastases can occur in bone, lung, mediastinum, and less commonly, the liver.[36]

Another study by Bensouda *et al.* concluded that metastases were found in only 5 to 7% of patients at initial diagnosis. These are mainly metachronous metastases found in evolution, usually within 3 years of treatment. The overall rate of metastasis is 25-30%. The occurrence of metastases is associated with the primary tumor (T), especially for lymph node involvement (N), and is most common in T3-T4 or N2-N3 tumors. The most frequent sites of metastases were bone (70-80%), followed by liver 30%, lungs, 18%), and, to a lesser extent, extra-cervical lymph nodes (axillary, mediastinal, pelvic, inguinal). Prognosis depends on location: hepatic and medullary involvement has a poor prognosis, while isolated bone metastases may be associated with prolonged survival. [37]

LMP-1 expression results

These results illustrate that EBV infection is the biggest risk factor in this NPC case, just like other endemic areas. Based on the literature by Khabir A *et al*., it was found that LMP-1 was frequently detected on NPC biopsy but with wide variation among tumors. Based on most reports from various parts of the world, approximately 50%-60% of NPC biopsies, LMP-1, can be visualized in most tumor cells using immunohistochemical techniques. LMP-1, which is highly expressed in NPC specimens, is thought to play a role not only in oncogenesis but also in maintaining the latent nature of the virus.[38]

The results of this study are in accordance with the study conducted by Hau *et al*., showing that increased LMP1 expression in NPC cells was associated with Id1 overexpression. Id1 (inhibitor of differentiation-1) is an activator of the -cell proliferation process.[39] Another study by Zheng *et al*. explained that LMP1 is an integral membrane protein containing a cytoplasmic amino terminus, six transmembrane domains, and a long cytoplasmic carboxy-terminal portion. LMP1 functions as a tumor necrosis factor receptor (TNFR) surrogate, activating several signaling pathways. LMP1 is functionally similar to CD40, a member of the tumor necrosis factor receptor superfamily, giving rise to B cell growth and differentiation signals. Two functional domains, namely C-terminal activation regions-1 (CTAR-1) and CTAR-2, in the cytoplasmic carboxyl terminus of LMP1, can activate the transcription factor NF-kB, which can lead to upregulation of antiapoptotic gene products. LMP1 plays an important role in the immortality of human B cells by activating some cellular signaling pathways, including NF-kB, JNK, JAK/STAT, p38/MAP, and Ras/MAPK. LMP1 alters several functional materials involved in tumor progression and invasion in human nasopharyngeal epithelial cells. The findings in this study indicated that LMP1 increased the transcription and expression of MMP-9 through NF-kB and AP-1, which was one of the mechanisms of LMP1 in mediating the invasion and metastasis of NPC cells. In addition, LMP1 also increased VEGF transcription and expression in NPC cell lines through the JAK3/STAT3 pathway. LMP2 was not involved in B cell transformation in vitro, but its expression showed an important role in

maintaining the virus in the body. In this study, it was stated that LMP1 is the main molecule in the pathogenesis of NPC; thus, disrupting LMP1 signaling becomes a promising strategy for targeted therapy in NPC.[40]

Gullo *et al*. explained that EBV-encoded latent membrane proteins; LMP1 is an integral membrane protein with oncogenic potential, encoded by the BNLF-1 gene (also known as the LMP1 gene) EBV. It can transform rodent cells and alter the phenotype of both lymphoid and epithelial cells. LMP1 is expressed in most NPC and is strongly suspected of having an important role in the pathogenesis and development of NPC, and its expression is associated with poor prognosis.[41] Linct *et al*. concluded that EBV has morphological properties (bound only) to nasopharyngeal epithelial and lymphocytes. The expression of LMP1 in carcinoma cells indicated that the origin of these cells came from the nasopharynx.[42]

The p53 expression results

Another study by Sheu *et al*. also detected 95% of the mutant p53 protein in the nucleus of tumor cells.[43] Similar results were also obtained in another study by Suharto and Harijadi, which stated that mutant p53 expression was positive in 89.8% of NPC cases.[44] Research by Taweevisit in Bangkok on 60 cases of NPC found that there was an overexpression of mutant p53 protein in 73% of cases. It indicated that the mutant p53 protein is closely related to NPC tumorigenesis. Most of the NPC showed mutant p53 overexpression, and the majority of these mutant p53 were wild-type mutant p53 (normal) which might be a response to EBV infection. The overexpression of mutant p53 in NPC is not caused by mutant p53 protein.[45]

This study is in line with the research results by Agaoglu *et al*., which argued that the immunohistochemistry-defined overexpression of mutant p53 was high. An overall immunoreactivity rate of 95% with strong immunoreactivity (>10%) in 65% of patients has been reported in Chinese patients. Among the total 97 samples, positive staining for mutant p53 protein was observed in 83 (85.5%) samples, while no staining was found in 14 (14.5%) cases. Immunoreactivity was observed in 62 (81.5%) primary nasopharyngeal biopsy specimens.[14] Zhang *et al*. explained that the mutant p53 pathway is one of the most important pathways regulating the cell cycle. Mutant p53 is activated in response to various internal and external stresses and increases the transcription factor p21 and its translation products. p21 induces G1 cell cycle arrest and DNA repair by inhibiting cyclin-dependent kinase-5 and increasing the expression of inducible growth arrest and DNA damage genes. When DNA damage is irreparable, mutant p53 induces apoptosis by increasing Bcl-2-associated protein X (BAX) expression and the formation of BAX homodimers. Mutations in the mutant p53 gene are among the most common genetic changes in human tumors. Approximately 50% of human tumors exhibit mutant p53 mutations.[46]

Furthermore, Sheu *et al*. concluded that the mutant p53 protein is closely related to NPC incidence. In most NPC studies, an increase in mutant p53 expression was found, and generally normal ("wild") mutant p53 was probably a reaction to Epstein-Barr virus infection because only 10% of NPCs had mutations in their mutant p53 protein.[43] Tejosukmono and Suharto explained that mutant p53 is thought to regulate the arrangement or function of the DNA replication-initiation complex. In the SV40 DNA replication-initiation process, the T antigen acts as a helicase to promote replication and binds to the DNA polymerase required to synthesize the SV 40 DNA. The mutant p53 protein can bind to the T antigen so that the activity of the T antigen as a helicase is inhibited. Moreover, there was no binding to DNA polymerase, so DNA synthesis did not occur. In cells that do not contain the SV40 virus or do not contain the T antigen, the mutant p53 protein binds to other compounds that are homologous to the T antigen.[13]

Another study by Lee *et al*. denoted that the stability and activation of mutant p53 and its transcriptional targets are critical for determining malignancy development or aversion. An important protein in regulating mutant p53 is MDM2, where MDM2 controls mutant p53 levels under non-stress conditions. The interaction between mutant p53 and MDM2 causes mutant p53 to be spread everywhere and eventually degraded; thus, blocking the interaction allows mutant p53 to be activated when a voltage is detected. One commonly used drug developed for this affinity is nutlin-3, widely used in cancer studies. The activation of the mutant p53 pathway has been described to induce cell cycle arrest or apoptosis via transcriptional targets such as p21 and Bax21. It suggests that not all mRNAs are equally translated into proteins as the regulation of transcription and translation varies under different conditions.[47]

In addition, Agaoglu *et al*. revealed that in head and neck cancer, high mutant p53 mutation rates were associated with tobacco consumption and poorer prognosis. Although point mutations in the mutant p53 gene were observed in nasopharyngeal cancer, the mutation rate was lower than in other tumors. On the other hand, mutant p53 can be easily detected with an extended half-life. Immunohistochemical studies have demonstrated significant overexpression of mutant p53 in NPC.[14]

Correlation between LMP-1 expression with regional lymph node involvement (N)

The results of this study align with other studies by Adam *et al*., revealing there was no significant relationship between LMP-1 expression with regional lymph nodes (N) with p 0.553.[15] In another study by Poida *et al*. in the Department of Anatomical Pathology, Faculty of Medicine, University of North Sumatra, 28 patients (82.4%) had lymph node diameters less than 6 cm, while 6 patients (17.6%) had different results. NPC patients with single lymph node enlargement were obtained as many as 25 people (73.5%) while those with multiple lymph node enlargements were 9 people (26.5%).[48]

Correlation between LMP-1 expression with distant metastases (M)

The results of this study showed that there was a negative correlation between the expression of LMP-1 with distant metastases (M) with the value of Sig. (2 tailed) = 0.019 (> 0.05), r count -0.370 > r table 0.312. It can be concluded that the higher the expression of LMP-1, the lower the incidence of distant metastases (M) in NPC patients. The results of this study are in line with research by Sarac *et al.,* which concluded that there was a significant relationship between LMP-1 expression and distant metastases (M) tumors with p 0.04[49]

Nakanishi *et al.* explained that the most common clinical symptom of NPC is the presence of cervical lymph node metastases represented as a neck mass. The first evidence for the relevance of LMP-1 regarding the metastatic properties of NPC is that LMP-1 induces matrix metalloproteinase (MMP)-9. While some studies have identified a positive correlation between LMP-1 expression and NPC metastatic status, other studies have failed to identify the link. These conflicting results may be due to the sample size and the method used to evaluate LMP-1. Furthermore, LMP-1 was shown to downregulate cell-cell adhesion and upregulate cell motility through its-1 and c-Met activation and ezrin expression. LMP-1 has also been shown to induce the expression of Mucin-1 (MUC-1), which plays an important role in tumor invasion and metastasis by countering cell adhesion.[50]

Correlation between p53 and lymph node involvement (N)

The results of this study are in line with other studies by Zhang *et al.* explained that the expression level of mutant p53 was significantly higher in NPC patients with lymph node metastases than those without lymph node metastases with p 0.001.[46]

Another study by Chow *et al.* explained that patients with high mutant p53 expression had significantly higher lymph node counts.[51] Another study by Aswarin *et al*. obtained different results showing no relationship between mutant p53 expression from metastatic lymph nodes in NPC. The results of the Mann-Whitney U test were p 0.706, so there was no significant relationship (p > 0.05) between the positive expression of mutant p53 protein in NPC and regional lymph nodes, namely N0, N1, N2, and N3. There was no significant difference between regional mutant p53 protein expression and lymph nodes in NPC. According to research, the greatest role of mutant p53 protein expression has been started early in tumor development, before the occurrence of spread to lymph nodes and distant metastases. In tumors with distant metastases, the mutant p53 protein's expression is small. Proteins that play a role in cell cycle control are suppressor genes and tumor oncogenes. The role of the mutant p53 protein in cell cycle regulation is to inhibit cell division, where the mutant p53 protein will trigger the p21 transcription process. An increase in p21 will cause the mutant p53 protein to inhibit all CDKs, while a non-functioning CDK will impact cyclins not to form complexes with CDKs; this affects the cell cycle stops. By triggering the cell division cycle again, the causative factor is the MDM-2 protein. The activity of this protein will suppress the activity of the mutant p53 protein. The low activity of the mutant p53 protein resulted in a decrease in p21 expression as a result that CDK was not inhibited so that a cycle would form a complex with CDK. The complex binding between CDK-cyclin will cause the cell cycle to continue. If a mutant p53 protein mutation occurs, the resulting protein is inactive and cannot trigger the formation of p21. Low p21 expression resulted in uninhibited CDK, and the cell division cycle continued. On the other hand, mutations in the mutant p53 protein result in the impaired activity of the BAX protein so that the pores in the mitochondrial membrane cannot open, and ultimately the cell does not undergo apoptosis.[52]

Correlation between p53 expression and the incidence of distant metastases (M)

The results of this study are different from other studies by Sawali *et al*., stating that NPC had a higher risk of distant metastases than other malignant head and neck tumors. With lymph node involvement, the high proliferation rate may explain its tendency to develop distant metastases. The incidence of isolated distant metastases is high, globally at 18%, and the risk increases with disease stage (47% for stage IVB), suggesting that locoregional treatment alone for locally advanced stages of the disease is inadequate. The lungs are the most common site of metastases, followed by bone and liver.[53]

Correlation between LMP-1 expression and p53 expression

The results of this study contradict another study by Shao *et al.*, who explained that the accumulation of mutant p53 in NPC was significantly correlated with LMP-1 overexpression.[54] Another study by Chou *et al*. showed that NPC cells had increased mutant p53 levels, and high levels of LMP-1 correlated with higher mutant p53 expression.[55] Lubis explained that LMP-1 inhibited the suppression effect of wild-type mutant p53, so tumor growth and progression occurred. LMP-1 was also able to defeat growth inhibition stimulated by wild-type p53 mutant. In addition, LMP-1 could cooperate with mutant p53 to induce the growth of NPC cells.[56]

Differences between LMP-1 expression and p53 expression

The results of this study are in line with other studies by Shao *et al*., who explained that Immunohistochemical analysis of NPC specimens showed a positive correlation between LMP-1 expression and mutant p53 expression.54 The results obtained indicated that the accumulation of mutant p53 in NPC was significantly correlated with LMP-1 overexpression. This study obtained the same results as the theory from the existing literature, namely NPC cells had increased mutant p53 levels and high levels of LMP-1 correlated with higher mutant p53 expression. LMP-1 inhibited the suppression effect of wild-type mutant p53, so tumor growth and progression occurred. LMP-1 was also able to defeat growth inhibition stimulated by wild-type p53 mutant. In addition, LMP-1 could cooperate with mutant p53 to induce the growth of NPC cells.[56]

The results of this study are different from the research by Asri A, denoting that there was a weak correlation between LMP-1 expression and mutant p53 expression (r 0.249) that had a positive pattern. Based on the results of statistical tests, there was a non-significant relationship between the expression of LMP-1 and the expression of mutant p53 (p 0.085).[57]

CONCLUSION

The expression levels of LMP-1 and mutant p53 were positive in NPC, especially in non-keratinizing carcinoma, undifferentiated type. Distant metastasis correlated with LMP-1, whereas lymph node involvement correlated with p53 mutant. Meanwhile, age and gender did not correlate with LMP-1 and mutant p53.

The results indicated that LMP-1 and mutant p53 expression could be used as determining factors in NPC prognosis and could be proposed as an alternative in the treatment of NPC by administering an LMP-1 inhibitor and mutant p53 as neoadjuvant chemotherapy.

CONFLICT OF INTEREST

There is no conflict of interest in this research.

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