Research

Correlation of LMP-1 expression with KRAS and Cyclin-D1 expressions in WHO type III NPC patients

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ABSTRACT

Background: Nasopharyngeal carcinoma (NPC) is a malignancy with pathologically and epidemiologically unique characteristics. The risk factors that are often associated with NPC are chronic EBV infection, environmental factors, and epigenetic changes. EBV infection expresses Latent Membrane Protein-1 (LMP-1) in NPC. The role of LMP-1 is to activate signaling pathways, including KRAS-RAF-MEK-ERK which induces transcription of cyclin D1 that contributes to cell proliferation. **Purpose:** To determine the correlation between LMP-1 expression and KRAS expression, LMP-1 expression with cyclin D1 expression, and KRAS expression with cyclin D1 expression in nasopharyngeal tissue of WHO type III NPC patients. **Method:** Analytical observational study with a cross-sectional approach involving 30 paraffin blocks of biopsy tissue from NPC patients who had not received radiotherapy or chemotherapy. Expression of LMP-1, KRAS, and cyclin D1 was examined with immunohistochemical staining method and calculated using manual counting by anatomical pathologists. **Result:** Statistical analysis of LMP-1 expression with KRAS expression showed an insignificant positive correlation (p=0.546) with a correlation coefficient (ρ=0.115). The LMP-1 expression with cyclin D1 expression showed an insignificant positive correlation (p=0.305) with a correlation coefficient (ρ=0.194). The KRAS expression with cyclin D1 expression showed an insignificant positive correlation (p=0.262) with a correlation coefficient (ρ=0.212). **Conclusion:** In WHO type III NPC tissue in the proliferative process, an increase in LMP-1 expression (53.4%±27.35%,) was followed by an increase in KARS expression (49.83%±22.83%) and D1 expression (42.27%±31.94%) as well as an increase in KRAS expression (42.27%±31.94%) followed by an increase in cyclin D1 expression (42.27%±31.94%) although not significant.

Keywords: NPC, LMP-1, KRAS, Cyclin D1, proliferation

ABSTRAK

Latar belakang: *Karsinoma nasofaring (KNF) adalah keganasan dengan karakteristik yang unik secara patologi maupun epidemiologi. Faktor risiko yang sering dikatkan dengan KNF yaitu infeksi kronik* Epstein-Barr Virus (EBV)*, faktor lingkungan serta perubahan epigenetik. Saat infeksi laten EBV, mengekspresikan* Latent Membrane Protein-1 (LMP-1) *pada KNF. Peran* LMP-1 *adalah mengaktifkan berbagai jalur pensinyalan yang salah satunya adalah* KRAS-RAF-MEK-ERK *serta meningkatkan fosforilasi serta translokasi nuklear EGFR yang pada akhirnya menginduksi transkripsi* cyclin D1 *yang berperan dalam proses proliferasi sel.* ***Tujuan:*** *Mengetahui korelasi antara ekspresi* LMP-1 *dengan ekspresi* KRAS*, ekspresi* LMP-1 *dengan ekspresi* cyclin D1*, dan ekspresi* KRAS *dengan ekspresi* cyclin D1 *pada jaringan penderita KNF WHO tipe III.* ***Metode:*** *Observasional analitik dengan pendekatan cross-sectional, melibatkan 30 blok parafin jaringan biopsi penderita KNF yang belum mendapat terapi berupa radioterapi maupun kemoterapi. Pemeriksaan ekspresi* LMP-1*,* KRAS *dan* cyclin D1 *menggunakan metode pewarnaan imunohistokimia dan hasilnya dihitung secara manual oleh ahli patologi anatomi.* ***Hasil:*** *Analisis statistik ekspresi* LMP-1 *dengan* KRAS *menunjukkan korelasi positif yang tidak signifikan (p =0,546) dengan koefisien korelasi (ρ =0,115). Ekspresi* LMP-1 *dengan* cyclin D1 *menunjukkan korelasi positif yang tidak signifikan (p=0,305) dengan koefisien korelasi (ρ=0,194). Ekspresi* KRAS *dengan* cyclin D1 *menunjukkan korelasi positif yang tidak signifikan (p=0,262) dengan koefisien korelasi (ρ=0,212).* ***Kesimpulan:*** *Di jaringan KNF WHO tipe III pada proses proliferasi, peningkatan ekspresi* LMP-1 *(53,4%±27,35%,) diikuti dengan peningkatan ekspresi*

KARS *(49,83%±22,83%) dan* cyclin D1 *(42,27%±31,94%,) begitu juga peningkatan ekspresi* KRAS *(42,27%±31,94%,) diikuti dengan peningkatan ekspresi* cyclin D1 *(42,27%±31,94) walaupun tidak signifikan.*

Kata kunci: *KNF,* LMP-1*,* KRAS*,* Cyclin D1*, proliferasi*

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) is one of the most common head and neck cancers. Based on data from GLOBOCAN in 2012, 87,000 new cases of NPC appeared each year with 61,000 new cases in male and 26,000 in female population, with 51,000 deaths. NPC was mainly found in men of reproductive age, the ratio of male and female was 2.18:1, with 60% of patients aged between 25-60. The highest incidence rate in the world was in the province of South China, which were 40-50 cases of NPC among 100,000 population.[1,2]

In Indonesia, NPC was the 4th highest malignancy after breast cancer, cervical cancer, and lung cancer. In Indonesia, the average prevalence recorded was 6.2/100,000 with 13,000 new cases per year.[3] Meanwhile, the Oncology Division of Otorhinolaryngology Head and Neck Surgery (OHNS), Dr. Saiful Anwar General Hospital (SAGH), Malang found that the average number of NPC patient visits was 37% of all patient visits to the OHNS oncology polyclinic during the period 2017 to 2019.[4–6]

Based on the histopathological description, the World Health Organization (WHO) classifies NPC into 3 subtypes: WHO type I keratinizing squamous cell carcinoma (keratinizing squamous cell carcinoma), WHO type II squamous without keratinization (non-keratinizing carcinoma), and WHO type III undifferentiated carcinoma (undifferentiated carcinoma).[7]

NPC is one of the malignancies associated with Epstein-Barr Virus (EBV). All cases of undifferentiated tumor type showed positive EBV.[8] In vitro, studies had found that persistent EBV infection in epithelial cells caused epithelial cells to become susceptible to carcinogenic exposure.[9]

Type III NPC has the highest EBV titer. EBV has tumorigenic potential because it has a unique set of latent genes, namely latent membrane proteins (LMP-1, LMP-2A, and LMP-2B) and EBV-determined nuclear antigens (EBNA1 and EBNA2).[10] Although EBV could cause latency in most individuals, only a few EBV infections progress to malignancy. This indicated that EBV was not sufficient to cause this malignancy. Environmental exposure and/or genetic risk factors also had a role in the pathogenesis of NPC.[11]

Latent Membrane Protein (LMP-1) can prevent apoptosis of EBV-infected cells by inducing anti-apoptotic proteins such as BCL-2, A20, and MCL-1.[12] The LMP-1 molecule encompasses a transmembrane domain and a carboxyl terminus containing the C-terminal Activating Region (CTAR) signaling domain. This transmembrane domain assists LMP-1 to bind to the host membrane where the CTAR region directly activates some signaling pathways including Nuclear Factorκ-B (NF-κB), Mitogen-Activated Protein Kinase (MAPK), and Phosphatidylinositol-3-Kinase. (PI3K). The importance of LMP-1 in tumorigenesis had been demonstrated in

various studies that reported inhibition of LMP-1 caused an increase in the sensitivity of tumor cells to chemotherapy. [13,14]

RAS is a member of the G protein family with intrinsic guanosine triphosphate (GTPase) activity involved in various cellular signal transduction pathways. Like other members of the RAS, KRAS bound to GTPases becomes activated and produces a series of downstream signaling cascades that then initiate cell growth, differentiation, proliferation, and cell survival. [15,16]

Active point mutations in the KRAS gene have been found at high rates in a wide variety of human tumors. For example, 90% of pancreatic cancers contain KRAS point mutations, as do 50% of colon and thyroid cancers.[17] The mutant RAS is insensitive to GAP and associated with GTP. It indicates permanent activation of downstream effectors, particularly the RAF- MEK-ERK pathway.[18]

Interestingly, KRAS gene mutations are rare in breast cancer but their upregulation has been found in 60% of tumors. The same situation had been observed in head and neck tumors, including NPC. Therefore, KRAS mutations did not appear to play a major role in tumors, but several studies had revealed that KRAS overexpression was a frequent event in these tumors (breast cancer and NPC).[17]

Cyclin D1 is involved in the regulation of cell development in the G1 phase and plays an important role in NPC tumorigenesis. It has been shown that NPC cells show overexpression of cyclin D1. Overexpression of cyclin D1 allows cells with damaged chromosomes and DNA to pass through the S phase without repair, and this increases the risk of cancer development.[12]

It is known that the role of LMP-1 in addition to preventing the apoptotic process by inducing anti-apoptotic proteins can also activate various signaling pathways, one of which is RAS-RAF-MEK-ERK. Besides, it increases phosphorylation and nuclear translocation of EGFR which in turn induces transcription of cyclin D1 that plays a role in the cell proliferation process. RAS mutations are not the same in every malignancy, but in the absence of mutations, KRAS still plays an important role in oncogenesis.

The purpose of this reasearch was to find the correlation between LMP-1 expression and KRAS expression, LMP-1 expression with cyclin D1 expression, and KRAS expression with cyclin D1 expression in nasopharyngeal tissue of WHO type III NPC patients.

METHOD

This research was an analytical observational study with a cross-sectional approach by examining the expression of LMP-1, KRAS, and cyclin D1 in nasopharyngeal tissue of patients with WHO type III NPC, using the immunohistochemical (IHK) method. Sampling used the quota sampling technique. The research samples were paraffin blocks of nasopharyngeal biopsy tissue from WHO type III NPC patients stored in the Dr. Saiful Anwar General Hospital (SARGH) Anatomical Pathology Laboratory, and their respective medical records which were stored in the SARGH outpatient medical record section, in the 2019-2020 period, and met the inclusion criteria.

The inclusion criteria were subjects who had not received treatment in the form of radiotherapy or chemotherapy.

Histopathological examination was carried out at the Anatomical Pathology Laboratory SARGH by the doctor on duty. It was fixated with 10% formalin buffer for 2 hours, dehydrated with 70%, 80%, 95% and absolute alcohol three times for 1.5 hours each. It was cleared with xylol three times, the first for 1 hour and the next for 1.5 hours each. It was infiltrated using liquid paraffin

twice, the first for 1.5 hours and the second for 2 hours, and cut with a rotary microtome. It was incubated at 50°C for 15 minutes and stained with Hematoxylin Eosin (HE). The results were examined microscopically.

Staining of LMP-1, KRAS, and cyclin D1 with immunohistochemistry was carried out at the Anatomical Pathology Laboratory, SARGH, Malang, with immunohistochemical examination techniques. Paraffin block preparation: the tissue was washed with Phosphate Buffered Saline (PBS), fixed with 10% formalin, dehydrated using graded alcohol (30%, 50%, 70%, 80%, 96%, and absolute). It was also cleared using xylol twice in 60 minutes. Infiltration used soft paraffin for 60 minutes at 480°C, it was blocked in hard paraffin in molds and left for a day and pasted. The specimen was cut 4-6 mm thick with a rotary microtome.

Mounting 5% gelatin on objects through deparaffinization process: slides were immersed in xylol twice, each for 5 minutes. Rehydration used graded alcohol (30%, 50%, 70%, 80%, 96% and absolute) for 5 minutes each, then it was rinsed with H2O for 5 minutes.

Immunohistochemical process against LMP-1, KRAS, and Cyclin D1: slide preparations were washed with PBS pH 7.4 and applied 3% H2O2 for 10 minutes. It was washed with PBS pH 7.4 for 5 minutes 3 times blocked using serum 2% Bovine Serum Albumin (BSA) for 60 minutes (1%). Incubation used primary antibodies LMP-1, KRAS and Cyclin D1 overnight at 40°C (1:100). It was washed with PBS pH 7.4 for 5 minutes 3 times and dropped with labeled secondary antibody and incubated for 1 hour (1:200). Washing using PBS pH 7.4 for 5 minutes 3 times. Dropping used SA-HRP (Strept Avidin Horseradish Peroxidase) for 40 minutes (1:500). It was washed with PBS pH 7.4 for 5 minutes 3 times and applied the chromogen for Horseradish Peroxidase (HRP), namely DAB (Diamino Benzidine). Rinsing used H2O and washing with PBS pH 7.4 for 5 minutes 3 times. Counter straining was done with Mayer Hematoxylin (Vision lab) for 10 minutes, washing with tap water. It was left until dry and mounting using Canada turpentine and object glass.

Patient characteristics were analyzed using descriptive statistics and presented in the form of a frequency distribution table. Analyzing the correlation between LMP-1 expression and KRAS in NPC used Pearson's test, and if the distribution was not normal using Spearman's test. Analyzing the correlation between LMP-1 expression and cyclin D1 expression in NPC used Pearson's test, if the distribution was not normal using Spearman's test. Analyzing the correlation of KRAS expression to cyclin D1 expression in NPC used Pearson's test, if the distribution was not normal using Spearman's test. Analyzing the effect of LMP-1 expression on cyclin D1 expression through KRAS expression in NPC using Path Analysis test (median path model).

RESULT

The general characteristics of the research subjects consisted of gender, age group, and employment status listed in Table 1. The ratio of the number of males and females in this research was 3.28:1. Based on the age group, the highest research subjects were the age group of 41-50 years, namely 13 subjects (43.3%), the lowest was the age group 21-30 years, and the age group 71-80 years with 1 subject each (3.3 % and 3.3%). In this research, it was found that the most types of work were farmers with 14 subjects (40%).

Table 1. Subject characteristics

General characteristic	N	%
Gender		
Male	23	76.7
Female	7	23.3
Age group		
21–30 years old	1	3.3
31–40 years old	5	16.7
41–50 years old	13	43.3
51–60 years old	6	20
61–70 years old	4	13.3
71–80 years old	1	3.3
Occupation		
Farmer	12	40.0
Vendors (vegetables, fruit,toys, etc.)	4	13.3
Carpenter	3	10.0
Employee	2	6.7
Chemical factory employee	1	3.3
Truck driver	1	3.3
Student	1	3.3
Housewife	6	20.0

The characteristics of the clinical stadium of the research subjects were presented based on T, N, and M referring to AJCC 2017. The most T values obtained were T2 of 14 subjects (46.7%), followed by T4 and T3 of 8 (26.7%) and 5 subjects (16.7%), respectively. While the highest N value was N3 of 16 subjects (53.3%) and the least was N0 of 2 subjects (6.7%). The number of distant metastases (M1) was 5 subjects (16.7%). The clinical-stage/stadium of NPC research subjects based on the 2017 AJCC was at most stage IVa of 15 subjects (50%).

Table 2. Clinical stadium of research subjects

Clinical stadium	N	%
T		
T1	3	10
T2	14	46.7
T3	5	16.7
T4	8	26.7
N		
N0	2	6.7
N1	4	13.3
N2	8	26.7
N3	16	53.3
M		
M0	25	83.3
M1	5	16.7
Stadium		
II	2	6.7
III	8	26.7
IVa	15	50
IVb	5	16.7

The expression of LMP-1, KRAS, and cyclin D1 in nasopharyngeal tissue of patients with WHO type III NPC were estimated with IHC examination.

On IHC examination, LMP-1 expression was in the form of brown color on the cell membrane and cytoplasm.[19] KRAS expression

was a golden-brown color appearance in the cytoplasm and cell membrane,[17] while the expression of cyclin D1 was a golden-brown color in the cell nucleus.[20] Expression LMP-1 had obtained an average of 53.4%±27.35%, a median of 65% of subjects which had the highest expression value 95% and the lowest expression value 2%. KRAS expression had obtained an average of 49.83%±22.83%, median 60%, subjects which had the highest expression value 80% and the lowest 10%. The average expression of cyclin D1 was 42.27% ±31.94%, median 35%, subjects with the highest expression value of 100% and the lowest of 0%. The expression of LMP-1, KRAS, and cyclin D1 was assessed quantitatively by manual calculation, assessed in 5 fields of view.

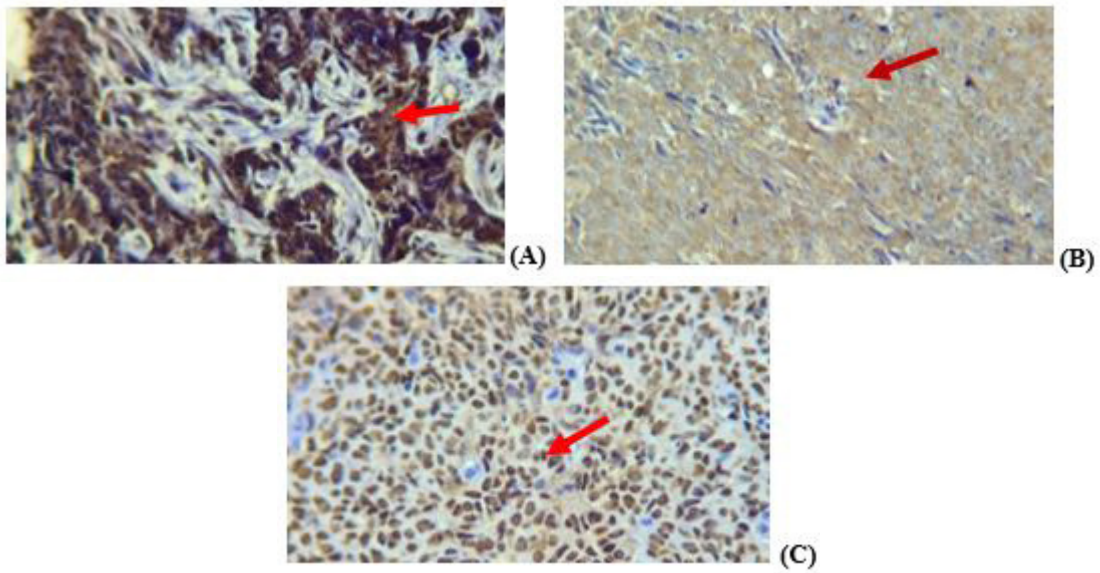

Figure 1. Expression of LMP-1, KRAS, and Cyclin D1. (A) The expression of LMP-1 in the cell membrane and cytoplasm (stained brown, arrows) was 95%; (B) Expression of KRAS in the cell membrane and cytoplasm (golden brown stained, arrows) was 80%; (C) The expression of cyclin D1 in the cell nucleus (golden brown stained, arrow) was 100%, the three expressions were assessed by manual calculation seen in 5 fields of view.

The correlation of LMP-1 Expression with KRAS in nasopharyngeal tissue in WHO type III NPC patients, was as follows: based on the normality test (Shapiro-Wilk), it was found that the expression of LMP-1 and KRAS had an abnormal distribution. Thus, to see the correlation between LMP-1 expression and KRAS expression in nasopharyngeal biopsy tissue in WHO type III NPC patients, the Spearman test was used. Spearman test results showed the correlation coefficient (ρ) of 0.115 with a significance value (p) of 0.546. It was greater than alpha 0.05 (p>0.05).

The correlation of KRAS expression with cyclin D1 in nasopharyngeal tissue in WHO type III NPC patients, was as follows: based on the normality test (Shapiro-Wilk), it was found that the expression of KRAS and cyclin D1 had an abnormal distribution. Thus, to see the correlation between KRAS expression and cyclin D1 expression in the biopsy tissue of WHO type III NPC patients, the Spearman test was used. Spearman test results showed the correlation coefficient (ρ) of 0.212 with a significance value (p) of 0.262. it was greater than alpha 0.05 (p>0.05).

The correlation of LMP-1 expression to cyclin D1 expression through KRAS expression in nasopharyngeal tissue in WHO type III NPC patients, was as follows: based on the normality test, it was found

that the expression variables of LMP-1, KRAS, and cyclin-D1 were not normally distributed. Hence, the correlation between LMP-1 expression and cyclin D1 expression through KRAS expression in this research could not be continued by path analysis. In addition, the correlation between the LMP-1 expression variable with the expression of KRAS and cyclin D obtained a p value >0.25 and it could not be included in the equation. The conditions for continuing Path Analysis were normal distribution, linear, and the significance of all variables ($p < 0.25$), if one of these conditions was not met, then it could not be continued in path analysis.

DISCUSSION

Twenty-three research subjects (76.7%) were males and 7 subjects (23.3%) were females,.The ratio of male: female was 3.28:1. Most of the subjects in this research were in the 41-50 years age group, namely 13 subjects (43.3%). It was similar with the study by Adham et al.[3], where 70.4% males and 26.9% females were found with a ratio of 2.4:1, and the most common age of patients with NPC was in the range of 41-50 years. Based on epidemiological data in China and Iran, the incidence of NPC began to increase at the age of 35 years with a peak in the fifth and sixth decades. In their study, it was found that the most occupation as farmers were 12 subjects (40%).[14] Research by Turkoz et.al.[21] revealed that an occupation that could increase risk factors was farmers because of frequent exposure to pesticides.

Patients with NPC might present with one or more symptoms associated with the location of the primary tumor, infiltration of structures around the nasopharynx, or cervical lymph node metastases. The symptom that most often caused sufferers to come for treatment was a lump in the neck. Distant metastases of NPC were generally rare with the most common sites being the lung, vertebrae, and liver. Patients usually came at an advanced stage when a lump in the neck appeared, nerve disturbances occurred, or distant metastases.[22] It was reported that >70% of patients were in an advanced stage when NPC was first diagnosed, this was because NPC was often occurred in a hidden anatomical location, and had a high metastatic rate.[23]

On imunnohistochemistry (IHC) examination, LMP-1 expression was in the form of a brown color appearing on the cell membrane and cytoplasm. LMP-1 expression was assessed quantitatively by manual calculation by Anatomical Pathologists in 5 fields of view. In this research, the average expression of LMP-1 was 53.4%±27.35%, the median was 65%; with the highest expression of 95% and the lowest of 2%. According to Sarac's research cited by Chen et al.[24], LMP-1 could be considered as positive if a chocolate stain was detected regardless of the expression value. In this research, all subjects were LMP-1 positive which was in accordance with the literature stating that all cases of NPC type III (undifferentiated) showed positive EBV, and various expression levels of LMP-1 were obtained.[9]

On IHC examination, KRAS expression was in the form of a golden-brown color appearance on the cytoplasm and cell membranes. KRAS expression was assessed quantitatively by manual calculation by Anatomical Pathologists in 5 fields of view. In this research, the average KRAS expression was 49.83%±22.83%, the median 60%; with the highest value of 80% and the lowest of 10%. The immunoreactive cut-off of KRAS was 20%, where <20% was the low expression and 20% was overexpression.[25] In this research, KRAS overexpression was found in 29 subjects (96.7%). Research by Ghazi et al.[26] found a 46% increase in KRAS expression in oral cancer patients. This indicated the involvement of KRAS in mitogenic signal transduction resulting

in increased proliferation and cell cycle regulation.[17]

On IHC examination, the expression of cyclin D1 was in the form of a golden-brown color appearance in the cell nucleus. Cyclin D1 expression was assessed quantitatively by manual calculation by Anatomical Pathologists in 5 fields of view. In this research, the average expression of cyclin D1 was 42.27%±31.94%, the median was 35%. The cut-off of the immunoreactive value of cyclin D1 was 10%. It meant that tumors with an expression of 10% were considered as the low expression, while >10% were said to be overexpressed.[27] In this research, 22 subjects (73.33%) with an expression >10%, it could be concluded that most of cyclin D1 were overexpressed. The frequency of overexpression of cyclin D1 from head and neck tumors was 20-68%.[28]

Based on the correlation test between LMP-1 expression and KRAS expression in the biopsy tissue of WHO type III NPC patients in this research using Spearman test and showed a correlation coefficient value (ρ=0.115) with a significance value (p=0.546) which was greater than 0.05, so it could be concluded that there was a statistically insignificant positive correlation between LMP-1 expression and KRAS expression in biopsy tissue of WHO type III NPC patients. It could be interpreted that the higher LMP-1 expression would be followed by an increase in KRAS expression, although it was not significant. In this research, it was found that all subjects had positive LMP-1 expression and KRAS overexpression in 29 subjects (96.7%); where several studies had revealed that KRAS overexpression was a frequent event in breast cancer and NPC.[17]

According to Tulalamba et al.[12], various pathways affected the proliferation process in NPC. The Mitogen Activated Protein Kinase (MAPK) pathway known as the RAS-RAF-MEK-ERK pathway played a role in transmitting signals from receptors on the cell surface to the nucleus through phosphorylation of various protein kinases. Zhang et al.[29] detected 17.1% of mutations in NPC where PI3CA, KIT, and RAS mutations were the most susceptible oncogenes to mutation. KRAS, HRAS, and NRAS mutations occurred in one-third of cancers that occurred in humans where KRAS mutations were the most common among the three. However, in their research, no mutations were found in KRAS and only 4.1% mutations were found in NRAS.

The MAPK pathway could be activated and increased by disturbances above the RAS, for example, overexpression or mutations in EGFR could activate KRAS. The relationship between KRAS and EGFR was the positive regulation of EGFR ligand expression mediated by KRAS. LMP1-CTAR1 activated EGFR and ERK through protein kinase C delta, which then triggered NPC cell motility and invasiveness through activation of the ERK-MAPK pathway. EGFR overexpression in NPC was reported to be high and found in up to 80% of cases; and this overexpression was found in advanced NPC.[12]

In this research, it was shown that there was a statistically insignificant positive correlation between KRAS expression and cyclin D1 expression in the biopsy tissue of WHO type III NPC patients. It could be interpreted that the higher the KRAS expression value, it would be followed by an increase in cyclin D1 expression although it was not significant. In a study conducted by Luangdilok et al.[30], in which NSCLC (Non-Small Cell Lung Cancer) patients with mutated KRAS had higher cyclin D1 overexpression than non-mutated KRAS. This might imply that KRAS mutations in NSCLC might induce overexpression of cyclin D1 outside the activity of the RAS-MEK-REK pathway. The presence of mutations in KRAS, allowed the KRAS protein to always be in an active state. It meant that it continued to transmit signals for cell growth, differentiation, and invasion.

Musgrove et al.[28] stated that many pathways regulated cyclin D1, including the PI3K pathway. PI3K affected cellular functions including proliferation. LMP-1 was a key effector of EBV-mediated nasopharyngeal cell transformation, directly activating PI3K which Akt phosphorylates, and activated several signaling pathways including p27 degradation, which resulted in cell cycle progression. Through the activity of Akt, c-Fos which encoded an oncogenic protein that bound to the c-Jun protein to form a transcription factor was AP-1. It was an important regulator for cell proliferation and survival in NPC. Mitogenic signals that induced the formation of cyclin D, would also induce the formation of cyclin E and two cyclin-dependent kinase inhibitors (CDKI), namely: p21cip1 and p27kip1. These two CDKIs were bound to cyclin D-Cdk4 but did not inhibit its kinase activity. The results showed that p21cip1 and p27kip1 were required for the formation and migration of cyclin D-Cdk4 by the cell nucleus. Both CDKIs were effective in inhibiting the activity of cyclin E-Cdk2.[30] In the study of Nurhidayat et al.[31] it was found that overexpression of cyclin D1 was found in 86.4% of radioresistant NPC patients.

There were limitations to this study. It only investigated the proliferative pathway involving LMP-1, KRAS, and cyclin D1 while there were many other pathways in the proliferation process in NPC where oncogenes in these pathways could affect research variables.

In conclusion, there was a statistically insignificant positive correlation between LMP-1 expression and KRAS expression, LMP-1 expression, and cyclin D1 expression, and KRAS expression with cyclin D1 expression in the nasopharyngeal tissue of WHO type III NPC patients.

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