Research

Correlation of LMP-1 expression with KRAS and IL-8 expression in NPC WHO type III

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ABSTRACT

Background: Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma originating from mucosal epithelium of the nasopharynx with complex disease progression. About 95% are caused by Epstein-Barr virus (EBV) infection which is characterized by the detection of viral gene product protein of Latent Membrane Protein-1 (LMP-1). Tumor growth and metastasis depend on the mechanism of angiogenesis. Interleukin-8 (IL-8) is a potent angiogenic factor and involved in the angiogenesis mechanism. Kirsten Rat Sarcoma (KRAS) is one of the proto-oncogenes that has an increased expression of more than 60% in NPC. The KRAS activation played a role in the modulation of IL-8 expression by triggering several important signaling pathways, which triggered neovascularization in the process of angiogenesis. **Purpose:** To determine the correlation between expression of LMP-1 with KRAS and IL-8, in mechanism of angiogenesis in NPC WHO type III. **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. Expressions of LMP-1, KRAS, and IL-8 were examined with immunohistochemistry (IHC) staining method, and calculated using manual counting by Anatomic Pathologists. **Result:** Statistical analysis of LMP-1 expression with KRAS showed an insignificant positive correlation (p=0.546), with a correlation coefficient (ρ=0.115). The KRAS expression with IL-8 showed an insignificant positive correlation (p=0.851), with a correlation coefficient (ρ=0.036). The LMP-1 expression with IL-8 showed a significant positive correlation (p=0.042), with a correlation coefficient (ρ=0.321). **Conclusion:** The increase in the expression of LMP-1 was followed with the increase in the IL-8 expression.

Keywords: NPC, LMP-1, KRAS, IL-8, angiogenesis

ABSTRAK

Latar belakang: *Karsinoma nasofaring (KNF) adalah karsinoma sel skuamosa yang berasal dari epitel mukosa nasofaring. Perkembangannya melibatkan hubungan yang kompleks. Sekitar 95% disebabkan oleh infeksi virus* Epstein-Barr (VEB) *yang ditandai dengan terdeteksinya protein produk gen virus, salah satunya yaitu* Latent Membrane Protein-1 (LMP-1). *Pertumbuhan dan metastasis tumor tergantung pada mekanisme angiogenesis.* Interleukin-8 (IL-8) *adalah faktor angiogenik yang kuat, dan terlibat dalam mekanisme angiogenesis.* Kirsten Rat Sarcoma (KRAS) *merupakan salah satu proto-onkogen yang mengalami peningkatan ekspresi lebih dari 60% pada KNF. Aktivasi* KRAS *memainkan peran dalam modulasi ekspresi* IL-8 *dengan memicu beberapa jalur sinyal penting, dan hal ini dapat memicu neovaskularisasi pada proses angiogenesis.* ***Tujuan:*** *Mengetahui korelasi antara ekspresi* LMP-1 *dengan ekspresi* KRAS *dan* IL-8 *dalam mekanisme angiogenesis KNF* WHO *tipe III.* ***Metode:*** *Penelitian observasional analitik dengan pendekatan potong lintang yang melibatkan 30 blok parafin jaringan biopsi penderita KNF yang belum mendapat pengobatan radioterapi maupun kemoterapi. Pemeriksaan ekspresi* LMP-1, KRAS, *dan* IL-8 *menggunakan 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* KRAS *dengan* IL-8 *menunjukkan korelasi positif yang tidak signifikan (p=0,851),*

dengan koefisien korelasi ρ=0,036. Ekspresi LMP-1 *dengan* IL-8 *menunjukkan korelasi positif yang signifikan (p=0,042), dengan koefisien korelasi ρ=0,321.* ***Kesimpulan:*** *Semakin tinggi ekspresi* LMP-1*, maka diikuti oleh tingginya ekspresi* IL-8.

Kata kunci: *KNF,* LMP-1, KRAS, IL-8, angiogenesis

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a squamous cell carcinoma originating from the poorly differentiated nasopharyngeal mucosal epithelium with a strong metastatic tendency to to regional lymph nodes and/or distant organs.[1] The development of NPC involves a complex relationship between genetic factors, Epstein-Barr virus (EBV) infection, and environmental involvement. Based on the World Health Organization (WHO) histological classification in 1991, NPC is divided into three subtypes, namely WHO type I (keratinizing squamous cell carcinoma), WHO type II (nonkeratinizing carcinoma), and WHO type III (undifferentiated carcinoma). The most common subtypes was undifferentiated carcinoma (WHO-III).[1,2]

In 2008, the fifth highest incidence and mortality due to cancer in Indonesia was caused by NPC. The incidence of new cases was higher in men than women with 9.4 new cases in men and 3.8 cases in women per 100,000 population. The mortality rate for NPC in men is 6.0 deaths and in women is 2.4 per 100,000 population.[3] Almost 80% of patients with NPC in Indonesia were diagnosed in the productive age of 30-59 years, with a tendency to increase in incidence with increasing age.[4] In the Regional General Hospital of dr. Saiful Anwar Malang, NPC ranked number one with the new cases in 2020 reaching 29% of all patient visits to the Oncology Special Polyclinic of the Ear, Nose and Throat Department.[5]

A total of 95% of NPC is associated with EBV infection which was evidenced by the detection of viral gene product proteins, such as Latent Membrane Protein-1 (LMP-1), Latent Membrane Protein-2 (LMP-2), Epstein-Barr Nuclear Antigen1-6 (EBNA1-6), and an increase in antibody titers IgA viral capsid antigen (VCA) and early-antigen (EA) proteins.[4] LMP-1 is the main oncogene in NPC. About 80%-90% of cell growth in NPC is influenced by LMP-1. LMP-1 has a basic role as a preventer of apoptosis, but LMP-1 also has other important functions in cancer development. Cells with positive LMP-1 put these cells at greater risk of metastases and faster disease progression.[6]

Apart from EBV infection, the genetic changes were involved in the development of NPC. In human bodies there are genes that have the potential to trigger cancer called proto-oncogenes. One of the proto-oncogenes main linked in cancer research is Ras oncogene. Overexpression of Ras has been reported in many cancers and one of them is NPC.[7] There are three Ras oncogenes, namely Harvey Rat Sarcoma (HRAS), Kirsten Rat Sarcoma (KRAS), and Neuroblastoma Rat Sarcoma (NRAS).[8,9] Of the three types of Ras, it is known that an increase in KRAS expression was found more than 60% in NPC.[10] The KRAS activation can trigger several important signaling pathways that regulate cancer cell development.[8]

In the process of tumor growth and metastasis, the role of angiogenesis is essential. Angiogenesis is the process of

forming new blood vessels from existing blood vessels, which is tightly regulated by proangiogenic and antiangiogenic factors.[11,12] The imbalance of these factors results in the formation of excess blood vessels and plays a role in pathological processes such as malignancy.[11] Angiogenesis is associated with the development of tumor metastases, and provides a significant poor prognostic assessment.[13]

Interleukin-8 (IL-8) otherwise known as CXCL8 is a pro-inflammatory cytokine. Pro-inflammatory cytokines have been associated with inflammatory diseases, especially chronic inflammation. Levels of interleukins (ILs) have been observed to be associated with advanced cancer in several types of cancer, and are poor prognostic markers for malignancy.[14] In addition, IL-8 is also a potent angiogenic factor involved in angiogenesis, tumor development, and cell invasion; thereby stimulating cancer cell growth and contributing to metastasis and recurrence.[15] The potentiality of IL-8 had been observed in many types of cancer, one of which is nasopharyngeal cancer.[14] IL-8 expression is regulated by activator protein and/or transcriptional activity mediated by nuclear factor-κB (NFκB), so that EBV infection is an important factor in IL-8 secretion.[16] KRAS activation plays a role inmodulation of IL-8 expression by triggering several important signaling pathways, and this can trigger neovascularization in the process of angiogenesis.[8,17]

The KRAS and IL-8 play a role in the development of cancer. The research on the correlation of LMP-1, KRAS and IL-8 expression in NPC was not yet available in Indonesia. Therefore, this research was conducted to determine whether the KRAS-IL-8 signaling pathway plays a role in the WHO type III NPC angiogenesis mechanism with positive LMP-1.

METHOD

This research was an analytical observational study with a cross-sectional approach by examining the expression of LMP-1, KRAS, and IL-8 in nasopharyngeal tissue of patients with WHO type III NPC, using the immunohistochemistry (IHC) method. Sampling used the quota sampling technique. The research samples were paraffin blocks of nasopharyngeal biopsy tissue from patients with WHO type III NPC stored in the Anatomic Pathology Laboratory of Dr. Saiful Anwar Regional General Hospital (SARGH), 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 i.e subjects who had not received treatment in the form of radiotherapy or chemotherapy.

The histopathological examination was carried out at the Anatomic Pathology Laboratory of SARGH by the doctor on duty. The samples were fixated with 10% formalin buffer for 2 hours. They were dehydrated with 70%, 80%, 95%, and absolute alcohol three times for 1.5 hours each. Clearing with xylol three times, the first for 1 hour, and the next for 1.5 hours each. Liquid paraffin was infiltrated twice, the first for 1.5 hours and the second for 2 hours, and then cut with a rotary microtome. They were incubated at 50°C for 15 minutes, and stained with Hematoxylin Eosin (HE). The results were examined microscopically.

Staining of LMP-1, KRAS, and IL-8 with IHC was carried out at the Anatomic Pathology Laboratory, SARGH, Malang, with immunohistochemical examination techniques. Paraffin block preparation: the tissue was washed with Phosphate Buffered Saline (PBS), fixed with 10% formalin solution, and rehydrated using graded alcohol solutions (30%, 50%, 70%, 80%, 96% and absolute). It was also cleared using xylol twice in 60 minutes. Infiltration using soft paraffin for 60 minutes at 480°C. It was blocked in

hard paraffin in molds, and kept in store for one 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 rinsed with H2O for 5 minutes.

Immunohistochemical process against LMP-1, KRAS, and IL-8: 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, then blocked using 2% Bovine Serum Albumin (BSA) for 60 minutes (which was diluted as 1%). Incubation used primary antibodies LMP-1, KRAS, and IL-8 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). Then, washed with PBS pH 7.4 for 5 minutes 3 times. Furthermore, dropped with SA-HRP (Strep Avidin Horse Radish Peroxidase) for 40 minutes (1:500). It was then washed with PBS pH 7.4 for 5 minutes 3 times, and applied the chromogen for Horseradish Peroxidase (HRP), namely DAB (Diamono Benzidine). Rinsed with H2O, and washed with PBS pH 7.4 for 5 minutes 3 times. Counterstaining was done with Mayer Hematoxylin (Vision lab) for 10 minutes, then washed with tap water. It was left until dry and mounting, then viewed under a microscope.

Patient characteristics were analyzed using descriptive statistics, and presented in the form of a frequency distribution table. Analyzing the correlation between LMP-1 and KRAS expression in NPC used Pearson's test, if the distribution was not normal using Spearman's test. Analyzing the correlation between LMP-1 and IL-8 expression in NPC used Pearson's test, if the distribution was not normal using Spearman's test. Analyzing the correlation of KRAS expression and IL-8 expression in NPC using Pearson's test, if the distribution was not normal using Spearman's test. Analyzing the effect of LMP-1 expression on IL-8 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 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 12 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, traveling)	4	13.3
Carpenter	3	10.0
Employee	2	6.7
Chemical factory employees	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 IL-8 in nasopharyngeal tissue of patients with WHO type III NPC were assessed quantitatively by Anatomic Pathologists, with immunohistochemical (IHC) examination. On IHC examination, LMP-1 expression was in the form of brown color on the cell membrane and cytoplasm. KRAS expression was golden-brown color appearance in the cell membrane and cytoplasm, while the expression of IL-8 was brown streaks in the cytoplasm. Expression LMP-1 had obtained an average of 53.4%±27.36%, 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%±22.84%, median 60%, with the highest expression value 80% and the lowest 10%. The average expression of IL-8 was 81.60% ±10.95%, median 82.5%, subjects with the highest expression value of 95% and the lowest of 49%.

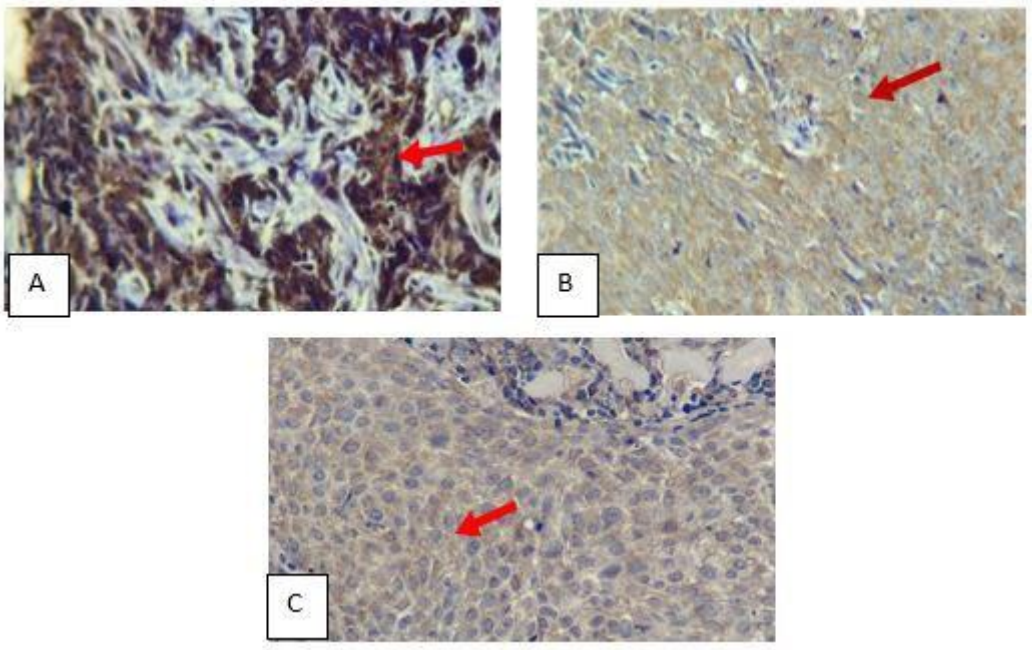

Figure 1. The expression of LMP-1, KRAS, and IL-8. (A) The expression of LMP-1 in the cell membrane and cytoplasm (stained brown, arrows) was 95%; (B) The expression of KRAS in the cell membrane and cytoplasm (golden brown, arrow) was 80%; (C) the expression of IL-8 in the cytoplasm (stained brown, arrow) was 80%, the three expression 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 tissue in WHO type III NPC patients using the Spearman test. 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 IL-8 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 IL-8 had an abnormal distribution. Thus, to see the correlation between KRAS expression

and IL-8 expression in nasopharyngeal tissue in WHO type III NPC patients using the Spearman test. Spearman test results showed the correlation coefficient (ρ) of 0.036 with a significance value (p) of 0.851. It was greater than alpha 0.05 (p>0.05).

The correlation of LMP-1 expression with IL-8 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 IL-8 had an abnormal distribution. Thus, to see the correlation between LMP-1 expression and IL-8 expression in nasopharyngeal tissue in WHO type III NPC patients using the Spearman test. Spearman test results showed the correlation coefficient (ρ) of 0.321 with a significance value (p) of 0.042. It was less than alpha 0.05 (p>0.05).

The correlation of LMP-1 expression to IL-8 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 IL-8 were not normally distributed. Hence, the correlation between LMP-1 expression and IL-8 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 IL-8 obtained 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

A total of 30 subjects consisted of 23 (76.7%) male and 7 (23.3%) female, with the ratio of male and female of 3.28:1. This distribution was in accordance with some literature which reported that NPC occurred 2-3 times more often in men than in women.[1] The results of this study were also similar to other study at the Department of ENT Health Sciences, Hasanuddin University Makassar for the 2011-2019 period, which observed a total of 68% of NPC patients were male. The high incidence in men could be caused by different lifestyles between men and women such as smoking habits, and occupational risks such as exposure to chemicals, steam, dust fumes, thereby increasing the risk of 2-6 times in the occurrence of NPC.[18,19]

Most of the subjects in this study were in the 41-50 year age group, consisted of 13 people (43.3%). This is in accordance with data from the Indonesian national cancer registry, where the age distribution of NPC patients was found to be the most at the age of 40-50 years. This finding is also in accordance with the research conducted by Melani and Sofyan at H. Adam Malik Hospital in Medan, that the most NPC cases was found in the 41-50 year age group.[18,19]

Most of the subjects in this study worked as farmers, as many as 12 subjects (40%). In a study conducted by Adoga et al.[20] in Nigeria regarding the relationship between environmental risk factors and lifestyle on the incidence of NPC and sinonasal cancer, it was found that the occupation that could increase the risk factor was farmers. This was associated with significant exposure to herbicides, pesticides and chemical fertilizers that contained nitrates, which were used to increase agricultural yields.

The clinical stage of the research subjects seen from the T, N, and M values referring to the 2017 AJCC, at most was stage IVa as many as 15 subjects (50%). In this study, almost all research subjects were in advanced stage (stage III-IV) as many as 28 subjects (93.3%). These results were in accordance with the literature which stated that most patients coming to a health facility were already in an advanced stage. This occurred because of the hidden location of the tumor in the

nasopharynx with early symptoms resembling upper respiratory tract infection.[21,22]

In a study conducted by Sarac[23] on WHO type III NPC, it was said that LMP-1 could be declared positive if a brown streak was detected regardless of its expression value. In this study, it was found that all subjects expressed LMP-1 which was characterized by the appearance of brown spots on the cytoplasm and/or cell membranes, so it was considered to be LMP-1 positive. This was consistent with the literature which stated that all WHO type III (undifferentiated carcinoma) NPC were associated with EBV infection, and various levels of LMP-1 expression were obtained from nasopharyngeal tissue biopsy examination.[24]

The KRAS expression by examination of IHC on nasopharyngeal tissue obtained a golden brown appearance on the cell membrane and or cell cytoplasm. Cut-off value the immunoreactivity of KRAS was 20%, where 20% expression was high expression and <20% expression was low expression.[25] In this study, it was found that 29 subjects (96.7%) had an expression value of >20%, so it could be said that KRAS experienced high expression in most of the subjects.

The expression of IL-8 by examination of IHC in nasopharyngeal tissue showed a brown appearance in the cytoplasm of the cells. The cut-off immunoreactivity value of IL-8 was 25%, which was high expression.[26] In this study, it was found that all subjects had expression values >25%, so that it could be said that IL-8 experienced high expression in all subjects of this study.

Based on correlation test LMP-1 expression with KRAS expression in this study using Spearman's test and showed a significance value (p) of 0.546 with a correlation coefficient (ρ) of 0.115, so it could be concluded that there was an insignificant correlation. This study found that all subjects had positive LMP-1 expression, and high KRAS expression in 29 subjects (96.7%). It was discussed in previous studies that high KRAS expression was a frequent finding in NPC and breast cancer.[8]

Epstein Barr virus (EBV) in NPC is in a latent period characterized by the detection of latent gene expression EBNA (1,2,3A, 3B, 3C,-LP), LMP (1, 2A, 2B), and EBER (1 and 2). These latent genes have their own role in the development of cancer. LMP-1 is the main oncogene that has been most studied and has a role in the growth of cancer cells.[6,27] Similar to LMP-1, LMP-2A exerts several oncogenic properties and transformation potential in NPC by modulating the expression of various oncogenes and tumor suppressor genes.[7] LMP-2A has the ability to activate the PI3K/Akt and β-catenin pathways, and has the ability to modulate NFkB and STAT signaling in EBV infected epithelial cells. These pathways are downstream targets of the RAS.[17,27] The presence of these genes allows an increase in KRAS expression without a major influence by LMP-1. However, until now, the role of other genes has not been widely studied, because it is suspected that the main oncogenic that plays the most role in NPC is LMP-1.

KRAS is a major regulator of tumor cell growth. KRAS activation can trigger several important pathways, such as PI3K-AKT and MEK-ERK where these two pathways play a role in the proliferative process, survival, and invasion.[8] In a study conducted by Bissada et al.[28], it was stated that the Ras oncogene is very involved in the pathogenesis of a cancer. Mutations of KRAS are reported to occur in 15-30% of all tumors. Mutations in the KRAS oncogene are most commonly found in pancreatic, colon and thyroid cancers. It seems that KRAS mutations play a small role in NPC, which is 4%. Several researchers disclosed that it was the increased expression of KRAS that was more common in NPC.[7,8,28]

Based on correlation test LMP-1 expression with IL-8 expression in nasopharyngeal biopsy tissue of WHO type III NPC patients, this study used the Spearman test and showed a significance value (p) of 0.042 with a correlation coefficient (ρ) of 0.321, so it could be concluded that there was a positive correlation which was significant, with a strong weak correlation between LMP-1 expression and IL-8 expression. Therefore, it could be interpreted that the higher the expression of LMP-1, the higher the expression of IL-8 would be. In this study, it was found that all subjects had positive LMP-1 expression and high IL-8 expression in 30 subjects (100%).

The results of this study were in accordance with the results of research by Yoshizaki et al.[29], who stated that there was a significant correlation between LMP-1 expression and IL-8 expression. These results indicated that LMP-1 might contribute to the mechanism of angiogenesis in NPC through the induction of IL-8. This was also supported by a study conducted by Liao et al.[15] which showed that high IL-8 expression contributed to metastasis and angiogenesis in NPC.

The LMP-1 is the main oncoprotein in EBV, and strongly contributes to the growth of NPC. Angiogenesis is the key to the process of growth, invasion, and metastasis of a tumor. The formation of neovascularization at the tumor site could increase the opportunity for tumor cells to enter the circulation so that it will increase the metastasis of a tumor. The angiogenic molecules that most contribute to the process of angiogenesis are VEGF, bFGF and IL-8. The molecule has been shown to influence the synthesis of micro-vessels.[29] Several clinical studies had shown a direct correlation of IL-8 expression in the formation of neo-angiogenesis in NPC. In addition, it could also be through the activation of the vascular endothelial growth factor (VEGF) pathway.[14,15]

Apart from KRAS, LMP-1 can induce IL-8 via other pathways such as PI3K and NFkB. LMP-1 induces IL-8 mainly through activation of NF-kB to take part in NPC angiogenesis, and only partially through AP-1. LMP-1 stimulates transcription of the IL-8 promoter 7-fold by activating NFkB and c-jun kinase in C33A cells.[29]

The LMP-1 has a tumor necrosis factor receptor-mimicking function that can induce NFkB and AP-1 activation. Many molecules are included in cell surface markers such as CD23, CD40, intercellular adhesion molecule-1, and the anti-apoptotic protein bcl-2 induced by LMP-1. However, LMP-1 does not always have the same effect on cellular signal transduction in epithelial cells. The NFB complex is a combination of various NFB protein families, and each different NFB protein family has varying effects on different NFB promoters. In Eliopoulos's study, quoted by Yoshizaki et al.[29], it was found that p38 mitogen-activated protein kinase and NFkB activation by LMP-1, and this contribute to the production of IL-8 in epithelial cells.

Based on the correlation test of KRAS expression with IL-8 expression in nasopharyngeal biopsy tissue in WHO type III NPC patients, which in this study using the Spearman test and showed a significance value (ρ) of 0.851 with a coefficient correlation value (ρ) of 0.36, so it could be concluded that there was an insignificant correlation between KRAS expression and IL-8 expression. This study also found that the expression of KRAS and IL-8 both experienced high expressions, although the correlation between those two was not statistically significant. These findings were in accordance with the outcomes of other researchers who found high expression of KRAS and IL-8 in head and neck malignancies including NPC.[8,10,29]

In a study conducted by Liao et al., it was shown that high IL-8 expression contributed to angiogenesis in nasopharyngeal cancer and

lymph node metastases. IL-8 increased the activity of matrix metalloproteinases 2 and 9 (MMP-2, 9) which could increase metastatic activity in malignancy.[14,15] Angiogenesis is associated with the development of tumor metastases and provides a significant poor prognostic assessment. Research by Sunaga et al.[26] also found a significant increase in IL-8 expression associated with KRAS activation due to oncogene mutations in lung cancer.

LMP-1 increases IL-8 through activation of the KRAS pathway, thereby triggering neovascularization in angiogenesis. Active KRAS triggers several important signaling pathways such as PI3K-AKT-NFkB and MEK-ERK-AP1 which will induce IL-8.[8,17] In Yoshizaki et al.[29] study it was found that LMP-1 induced IL-8 mainly through NFkB activation and partly through AP-1. Both pathways could be affected by the activation of KRAS. Meanwhile, LMP-1 itself could directly activate the NFkB pathway, or through PI3K-AKT activation, which then would induce IL-8.[30]

In addition, LMP-1 also activates the Wnt protein signaling pathway, which is a homeostasis guard protein, namely β-catenin. Continuous stimulation by Wnt will cause accumulation of β-catenin in the cytoplasm, which then undergoes translocation into the nucleus. This increase in β-catenin stimulation will increase Interleukin-8 as an angiogenic factor in NPC so that it will cause neovascularization and will facilitate the invasion of NPC cells into the surrounding area.[30]

In conclusion, there was a significant positive correlation between the expression of LMP-1 and IL-8 in the nasopharyngeal tissue of patients with WHO type III NPC. The increase in the expression of LMP-1 was followed with the increase in the IL-8 expression.

We acknowledge that this study was limited in evaluating specific pathway involving LMP-1, KRAS and IL-8 in angiogenesis, while other oncogenes might confound our finding, Thus, further research is needed to evaluate other pathways involvement in angiogenesis.

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REFERENCE

1. Wei WI, Chua DTT. Nasopharyngeal Carcinoma. In: Johnson JT, Rosen CA, editors. Bailey's Head and Neck Surgery Otolaryngology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p. 1875–97.
2. Usman HA, Soehartono, Rahayu P. Histopatologi. In: Farhat, Adham M, Dewi YA, Indrasari Sagung R, editors. Karsinoma Nasofaring. Jakarta: Penerbit Buku Kedokteran EGC; 2019. p. 29–38.
3. Sudiono J, Hassan I. DNA Epstein-Barr Virus (EBV) Sebagai Biomaker Diagnosis Karsinoma Nasofaring. Dent J (Majalah Kedokt Gigi). 2013; 46(3):140.
4. Savitri E, Haryana MS. Expression of Interleukin-8, Interleukin-10 and Epstein-Barr Viral-Load as Prognostic Indicator in Nasopharyngeal Carcinoma. Glob J Health Sci. 2015; 7(3):364–72.
5. SMF IK THT-KL. In: Laporan Tahunan Poliklinik THT-KL. Malang: Rumah Sakit Umum dr.Saiful Anwar; 2020.
6. Tsao SW, Tsang CM, Lo KW. Epstein-Barr Virus Infection and Nasopharyngeal Carcinoma. Philos Trans R Soc B Biol Sci. 2017; 372(1732).

7. Tsang CM, Lui VWY, Bruce JP, Pugh TJ, Lo KW. Translational Genomics of Nasopharyngeal Cancer. Semin Cancer Biol. 2019; 61:84–100.

8. Deng M, Tang H, Zhou Y, Zhou M, Xiong W, Zheng Y, et al. Mir-216b Suppresses Tumor Growth and Invasion by Targeting KRAS in Nasopharyngeal Carcinoma. J Cell Sci. 2011; 124(17):2997–3005.

9. Rajasekharan SK, Raman T. Ras and Ras Mutations in Cancer. Cent Eur J Biol. 2013; 8(7):609–24.

10. Hunt JL, Barnes L, Lewis JS, Mahfouz ME, Slootweg PJ, Thompson LDR, et al. Molecular diagnostic alterations in squamous cell carcinoma of the head and neck and potential diagnostic applications. Eur Arch Otorhinolaryngol. 2014; 271:211–23.

11. Amini A, Moghaddam SM, Morris DL, Pourgholami MH. The Critical Role of Vascular Endothelial Growth Factor in Tumor Angiogenesis. Curr Cancer Drug Targets. 2012; 12(1):23–43.

12. Hicklin DJ, Ellis LM. Role of the Vascular Endothelial Growth Factor Pathway in Tumor Growth and Angiogenesis. J Clin Oncol. 2005; 23(5):1011–27.

13. Gallo O, Franchi A, Magnelli L, Sardi I, Vannacci A, Boddi V, et al. Cyclooxygenase-2 Pathway Correlates with VEGF Expression in Head and Neck Cancer. Implications for Tumor Angiogenesis and Metastasis. Neoplasia. 2001; 3(1):53–61.

14. Zarogoulidis P, Katsikogianni F, Tsiouda T, Sakkas A, Katsikogiannis N, Zarogoulidis K. Interleukin-8 and Interleukin-17 for Cancer. Cancer Invest. 2014; 32(5):197–205.

15. Cheng D, Kong H, Li Y. Prognostic Value of Interleukin-8 and MMP-9 in Nasopharyngeal Carcinoma. Eur Arch Otorhinolaryngol. 2014; 271(3):503–9.

16. Long X, Ye Y, Zhang L, Liu P, Yu W, Wei F, et al. IL-8, a Novel Messenger to Cross-Link Inflammation and Tumor EMT via Autocrine and Paracrine Pathways (Review). Int J Oncol. 2016; 48(1):5–12.

17. Sparmann A, Bar-Sagi D. Ras-Induced Interleukin-8 Expression Plays A Critical Role in Tumor Growth and Angiogenesis. Cancer Cell. 2004; 6(5):447–58.

18. Dawolo AP, Utama DS, Kasim BI. Profil Klinis Karsinoma Nasofaring di Departemen THTKL RSUP Dr. Mohammad Hoesin Palembang. 2014-2015. Maj Kedokt Sriwij. 2017; 49(1):1–9.

19. Djufri NI, Pieter NAL. Epidemiologi. In: Farhat, Adham M, Dewi YA, Indrasari SR, editors. Karsinoma Nasofaring. Jakarta: Penerbit Buku Kedokteran EGC; 2019. p. 5–17.

20. Adoga AA, Kokong DD, Nimkur TL, Ma'an ND. Environmental and Life-Style Related Risk Factors for Sinonasal and Nasopharyngeal Malignancies among a Prospective Cohort in Jos, Nigeria. Int J Otolaryngol. 2018; 2018:1–6.

21. Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, et al. Nasopharyngeal Carcinoma in Indonesia: Epidemiology, Incidence, Signs, and Symptoms at Presentation. Chin J Cancer. 2012; 31(4):185–96.

22. Tabuchi K, Nakayama M, Nishimura B, Hayashi K, Hara A. Early Detection of Nasopharyngeal Carcinoma. Int J Otolaryngol. 2011; 2011:1–6.

23. Sarac S, Akyol MU, Kanbur B, Poyraz A, Akyol G, Yilmaz T, et al. Bcl-2 and LMP1 Expression in Nasopharyngeal Carcinomas. Am J Otolaryngol - Head Neck Med Surg. 2001; 22(6):377–82.

24. Morris MA. Cancer-Associated Fibroblasts in Undifferentiated Nasopharyngeal Carcinoma: A Putative Role for the EBV-Encoded Oncoprotein, LMP1. Pathogens. 2019; 9(1):1–11.

25. Essakly A, Loeser H, Kraemer M, Alakus H, Chon SH, Zander T, et al. PIK3CA and KRAS Amplification in Esophageal Adenocarcinoma and their Impact on the Inflammatory Tumor Microenvironment and Prognosis. Transl Oncol [Internet]. 2020; 13(2):157–64. Available from: https://doi.org/10.1016/j.tranon.2019.10.013

26. Sunaga N, Kaira K, Tomizawa Y, Shimizu K, Imai H, Takahashi G, et al. Clinicopathological and Prognostic Significance of Interleukin-8 Expression and its Relationship to KRAS Mutation in Lung Adenocarcinoma. Br J Cancer. 2014; 110(8):2047–53.

27. Young LS, Dawson CW. Epstein-Barr Virus and Nasopharyngeal Carcinoma. Chin J Cancer. 2014; 33(12):581–90.

28. Bissada E, Abboud O, Abou Chacra Z, Guertin L, Weng X, Nguyen-Tan PF, et al. Prevalence of K-RAS Codons 12 and 13 Mutations in Locally Advanced Head and Neck Squamous Cell Carcinoma and Impact on Clinical Outcomes. Int J Otolaryngol. 2013; 2013:1–6.

29. Yoshizaki T, Horikawa T, Qing-chun R, Wakisaka N, Takeshita H, Furukawa M, et al. Induction of Interleukin-8 by Epstein-Barr Virus Latent Membrane Protein-1 and its Correlation to Angiogenesis in Nasopharyngeal Carcinoma. Clin Cancer Res. 2001; 7(7):1946–51.

30. Soehartono, Rahaju P. Patomekanisme. In: Farhat, Adham M, Dewi YA, Indrasari SR, editors. Karsinoma Nasofaring. Jakarta: Penerbit Buku Kedokteran EGC; 2021. p. 39–50.