Evaluation of matrix metalloproteinase-9 expressions in nasopharyngeal carcinoma patients

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Abstract. Nasopharyngeal carcinoma (NPC) is one of head and neck cancer with a poor prognosis because of the position of the tumor adjacent to the skull base and vital structures. Degradation of extracellular matrix that will cause tumor cells to invade surrounding tissues, vascular or lymphatic vessels. One that plays a role in the extracellular matrix degradation process is matrix metalloproteinase-9 (MMP-9). MMP-9 plays a role in tumor invasion process, metastasis and induction of tumor tissue vascularization. To determine the expression of MMP-9 in patients with nasopharyngeal carcinoma, a descriptive study was conducted by examining immunohistochemistry MMP-9 in 30 NPC tissues that had never received radiotherapy, chemotherapy or combination. Frequency distribution of NPC patient mostly in the age group 41-50 years old and 51-60 years were nine people (30.0%); men (73.3%) and non-keratinizing squamous cell carcinoma (53.3%) histopathology type. The overexpression of MMP-9 in patients with nasopharyngeal carcinoma were mostly found in advance stage.

1. Introduction
Nasopharyngeal carcinoma (NPC) is 10 of most common cancers in Indonesia in 2004-2006 and continued to increase the number of patients during the period. Almost all patients came at an advanced stage, even with poor general circumstances. In Haji Adam Malik General Hospital Medan recorded that, from 335 patients during 2006-2010, 82.7% of patients diagnosed in the advanced stage. NPC is one of head and neck cancer with a poor prognosis because of the position of tumors adjacent to the skull base. NPC has a high potential of invading and metastasizing compared with head and neck cancer. Approximately 90% of patients with NPC showing cervical lymph node metastasis. NPC mortality is associated with tumor metastasis compared to primary tumors. The invasion and metastasis of tumor cells is a complex cascade one of which involves the secretion of proteolysis enzymes by tumor cells or host cells where the substrate is an extracellular matrix component. Matrix metalloproteinase (MMP) is zinc-dependent endopeptidase family that regulates the integrity and composition of the extracellular matrix. MMP-9 is one of MMP subgroup that has the capability in collagen type IV and V molecules degradation as well as gelatin, a major component of basal membrane.
2. Method
This descriptive study was conducted at the ENT Department of Haji Adam Malik General Hospital Medan. All samples were gathered by consecutive sampling. Data on sex, age, histopathology type, primary tumor (T), lymph node (N), and clinical stage were taken from examination. Immunohistochemical examination for MMP-9 expression was performed in Pathology Anatomy Department. All data were presented in tabular form.

3. Result
Thirty samples with various histopathologic types were examined by immunohistochemical staining to assess MMP-9 expression. In age group, 41-50 years and 51-60 years had the highest frequency distribution (30.0%), male (73.3%), and non-keratinizing squamous cell carcinoma (53.3%). Overexpression of MMP-9 was found in 10 NPC samples (33.3%), non-keratinizing squamous cell carcinoma (70%), primary tumor T3 of 4 samples (40.0%), lymph node metastasis N3 of 4 samples (40%) and stage in group IV of 6 samples (60%).

Table 1. Distribution of the characteristic frequency of research subjects.

| Characteristic  | N  | %   |
|----------------|----|-----|
| Age (year)     |    |     |
| ≤ 20           | 1  | 3.3 |
| 21 – 30        | 1  | 3.3 |
| 31 – 40        | 5  | 16.7|
| 41 – 50        | 9  | 30.0|
| 51 – 60        | 9  | 30.0|
| > 60           | 5  | 16.7|
| Sex            |    |     |
| Male           | 22 | 73.3|
| Female         | 8  | 26.7|
| Histopathological type |    |     |
| Keratinizing SCC | 1  | 3.3 |
| Non-Keratinizing SCC | 16 | 53.3|
| Undifferentiated carcinoma | 13 | 43.4|

Table 2. Distribution of histopathology, primary tumors, lymph nodes, and clinical stage in NPC based on MMP-9 expression.

| Characteristic      | MMP-9 Expression | Overexpression | Negative | %   |
|---------------------|------------------|----------------|----------|-----|
| Histopathological type |                  |                |          |     |
| Keratinizing SCC    | 0                | 0.0            | 1        | 5.0 |
| Nonkeratinizing SCC | 7                | 70.0           | 9        | 45.0|
| Undifferentiated carcinoma | 3            | 30.0           | 10       | 50.0|
| Primary Tumor (T)   |                  |                |          |     |
| T1                  | 2                | 20.0           | 5        | 25.0|
| T2                  | 1                | 10.0           | 4        | 20.0|
| T3                  | 4                | 40.0           | 3        | 15.0|
| T4                  | 3                | 30.0           | 8        | 40.0|
| Lymph Node (N)      |                  |                |          |     |
| N0                  | 0                | 0.0            | 1        | 5.0 |
| N1                  | 3                | 30.0           | 5        | 25.0|
| N2                  | 3                | 30.0           | 5        | 25.0|
| N3                  | 4                | 40.0           | 9        | 45.0|

Clinical Stage
4. Discussion

The aim of this study is to know the expression of MMP-9 in nasopharyngeal carcinoma, where the highest frequency distribution of nasopharyngeal carcinoma was found in the age group 41-50 years and 51-60 by nine peoples (30.0%) respectively. Malignancies are common in the elder (over 40 years) because the immune system and DNA repair mechanisms are poorly functioning. If this DNA repair mechanism fails in functioning, then the DNA gene mutations that have occurred will lead to uncontrolled cell growth.\(^{[12]}\) In gender, the male has the ratio of 3:1 compared to the female. Exposure to steam, dust fumes and chemical gases in the workplace increases the risk of NPC 2-6 times, while exposure to formaldehyde increases risk 2-4 times.\(^{[13]}\)

This study found the type of histopathology of patients with nasopharyngeal carcinoma is the type of non-keratinizing squamous cell carcinoma by 16 samples (53.3%).

![Figure 1. Left: Immunohistochemical staining of MMP-9 on NPC tissue with weak intensity (400x magnification). Right: Staining with strong intensity (400x magnification).](image)

The overexpression of MMP-9 was found in 10 of 30 NPC samples (33.3%). This is thought to be due to extranodal lymphoma T / NK cell type only a small amount of stromal element such as fibroblasts compared with carcinoma. Therefore MMP-9 is only slightly expressed. Increased expression of MMP-9 affects the ability of tumor cells to digest or damage the barrier of tissue, especially the basement membrane of the blood vessels.\(^{[10]}\) Overexpression of MMP-9 in NPC is thought to play a role in tumor growth by triggering angiogenesis and spreading local invasion and metastasis by degrading the extracellular matrix.\(^{[7]}\) Therefore, MMP-9 overexpression can be used as a marker for invasive processes and metastases of a carcinoma.\(^{[10]}\)

Overexpression value of MMP-9 in this study was mostly found in the non-keratinizing squamous cell carcinoma by seven samples (70.0%). EBV has an affinity with lymphocytes in the upper airway. During latent infection, EBV produces several types of proteins. The nasopharyngeal cells produce LMP1 and EBNA1.\(^{[14]}\) Besides, MMP is also involved in the production of VEGF. LMP-1 can also reduce certain TIMP expression.\(^{[15]}\)

MMP-9 overexpression is most common in T3 of 4 tissues of nasopharyngeal carcinoma (40.0%). Overexpression of MMP-9 in NPC accelerates tumor growth by affecting angiogenesis and improves local invasion and metastasis by degrading the extracellular matrix. This indicates that MMP-9 plays a role in the progressive progression of NPC tumor invasion and metastasis. Liu\(^{[7]}\) research on the NPC network showed an overexpression of MMP-9 in 85/116 NPC tissue with T1-2 and 42/46 T3-4 tissues. Angulo\(^{[11]}\) received elevated MMP-9 expression through RT-PCR examination in bladder cancer patients with T3-4 based on AJCC 2006.

The MMP-9 expression is related to the ability of tumor cells to enter the blood vessels and lymph. This may be due to the ability of MMP-9 to degrade type IV collagen, which is an important
component of the basement membrane. The increase of MMP-9 expression will facilitate cancer cells to penetrate the vessel membrane and then enter the bloodstream or metastasis to the lymph nodes. Increased MMP-9 expression was associated with lymph node metastasis.\cite{13}

Overexpression of MMP-9 was more common in stage III-IV of 9 samples (90.0%) tissues This result consistent with Liu\cite{8} who received MMP-9 more overexpressed at stage III-IV than stage I-II.

5. Conclusion
The overexpression of MMP-9 in patients with nasopharyngeal carcinoma most found in advance stage. It is necessary to conduct a follow-up study using a comparison / control to determine the relationship of MMP-9 expression to NPC clinicopathology regarding the role of MMP-9 in NPC process that can be used to provide optimal therapy.

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