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Analysis of Bacterial Colonization in Nasopharyngeal Carcinoma Patients

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Abstract. Nasopharyngeal carcinoma (NPC) is carcinoma originating from epithelial cells lining the nasopharynx. This epithelial surface is the entry point for bacteria. Bacterial colonization of the nasopharyngeal consists of pathogenic and commensal bacteria which when the immune system declines it will cause other systemic diseases. This can trigger chronic inflammation in the upper respiratory tract. Bacterial colonization of the nasopharynx can be influenced by several factors such as agents (microorganisms), host and the environment. The aim of this study is to analyze the occurrence of bacterial colonization in 30 patients with nasopharyngeal carcinoma at H. Adam Malik Hospital and Universitas Sumatera Utara Hospital, Medan, Indonesia. Nasopharyngeal swab specimen was isolated in microbiology laboratory, University of Sumatera Utara. Bacteria were identified by culture. The result showed of the 30 subjects, the most common age group was between 46 and 65 years of age (66.7%) with males as the majority (86.7%) and the most common histopathological type was non-keratinizing squamous cell carcinoma (76.7%). The most common bacterial colonization profile found in patients' cultures was *Staphylococcus aureus* in 12 cultures (36.36%). There were no positive correlation found for age, gender, smoking and histopathology type with bacterial colonization in patients with nasopharyngeal carcinoma.

Keyword: NPC, bacterial colonization, *Staphylococcus aureus*

Abstrak. Karsinoma nasofaring adalah karsinoma yang berasal dari sel epitel yang melapisi nasofaring. Permukaan epitel ini merupakan jalan masuk untuk bakteri. Kolonisasi bakteri pada nasofaring terdiri dari bakteri patogen dan komensal dimana pada saat sistem imun tubuh menurun maka akan menimbulkan penyakit sistemik lainnya. Kolonisasi bakteri pada nasofaring dapat dipengaruhi oleh beberapa faktor seperti agen (mikroorganisme), host dan lingkungan. Tujuan penelitian ini adalah untuk menganalisa terjadinya kolonisasi bakteri pada 30 pasien karsinoma nasofaring di RSUP H. Adam Malik dan RS Universitas Sumatera Utara, Medan Indonesia. Pemeriksaan spesimen swab dilakukan di laboratorium mikrobiologi di Universitas Sumatera Utara. Bakteri diidentifikasi dengan metoda kultur. Hasil penelitian menunjukkan bahwa dari 30 subjek, kelompok usia adalah antara 46-65 tahun (66,7%) dengan mayoritas adalah laki-laki (86,7%) dan tipe histopatologi yang paling umum adalah non-keratinizing squamous cell carcinoma (76,7%). Profil kolonisasi bakteri yang paling banyak ditemukan dalam kultur bakteri pasien dengan karsinoma nasofaring adalah *Staphylococcus aureus* dalam 12 kultur (36,36%). Tidak ada korelasi positif yang

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ditemukan untuk usia, jenis kelamin, merokok dan jenis histopatologis dengan kolonisasi bakteri pada pasien dengan karsinoma nasofaring..

Kata Kunci: karsinoma nasofaring, kolonisasi bakteri, *Staphylococcus aureus*

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1 Introduction

Nasopharyngeal carcinoma (NPC) is one type of cancer that is difficult to detect early. It is a tumour arising from the epithelial cells that cover the surface and line the nasopharynx [1]. This epithelial surface provides the most important entrance for pathogenic microbes. Epithelial defences are very important in the oral cavity and respiratory tract, where exposure from bacteria, fungi and viruses occur continuously [2]. Squamous cell carcinoma arises as a necrotic ulcerative lesion, usually surrounded by an inflammatory reaction. Necrotic tissue is a good medium for bacterial growth [3].

Bacterial colonization of the nasopharyngeal mucosa consists of pathogenic and commensal bacteria [4], others may be opportunistic, where when the immune system declines it will cause other systemic diseases [2], this can trigger chronic inflammation in the upper respiratory tract [5].

Pathogenic bacterial species found in the nasopharynx include *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Neisseria meningitidis* [4]. Bacterial colonization of the nasopharynx can be influenced by several factors that are interrelated to one another such as agents (microorganisms), host and environment [6].

Based on the literature search above, there has not been found a comprehensive study of nasopharyngeal bacterial colonization in NPC. Bacterial colonization in NPC patients with low immune system has the potential to easily develop into pathogenic bacteria, so researchers were interested in conducting this research to analyze the occurrence of bacterial colonization in patients with NPC.

2 Research Methods

This cross-sectional study was conducted in Haji Adam Malik General Hospital and University of Sumatera Utara Hospital Medan, Indonesia from September, 2018 until March, 2019. Thirty patients diagnosed with NPC were enrolled by doing anamnesis, physical examination, imaging studies, and histopathological examination by Otorhinolaryngologist. The samples had to fulfil the inclusion criteria including no antibiotic therapy in the past 2 weeks and no symptoms of respiratory tract infection. Patients agree to participate in the research and fill the informed

consent. This study also had been approved by the Health Research Ethical Committee of Medical Faculty, University of Sumatera Utara, Medan, Indonesia with No: 461/TGL/KEPK FK USU-RSUP HAM/2018.

2.1 Sample Collection

Nasopharyngeal swab specimens we collected using a flexible nasopharyngeal swab. Swabs were immediately placed in Amis Media and transported to the Microbiology laboratory at University of Sumatera Utara where microbiological tests were conducted.

2.2 Microbiological Assay

Quantitative culture assessment and bacterial identification were carried out using the Streak plate technique in accordance with standard microbiological laboratory procedures. Primary isolation was carried out on three pieces of microbiology media (blood agar media, chocolate agar media, and MacConkey media). The media was incubated and a colony was formed. The media was incubated at 35°C for 48 hours and formed colonies were observed. Colony growth can be seen after 24 hours to identify what colonies were growing in the three agar media [7].

Gram staining was done to determine the type of gram-positive or gram-negative in the growing colonies. Biochemical tests were carried out to identify the species of bacteria encountered.

2.3 Statistical Analysis

The statistical analysis was done by using SPSS software. Demographic data were listed in a univariate variable. Then, bivariate analysis was performed using Fisher exact test (χ^2) test to determine correlation found for age, gender, smoking and histopathology type with bacterial colonization in patients with NPC. Statistical significance was set at $p < 0.05$.

3 Results

3.1 Characteristics of Patients

There were 30 patients with NPC involved in this study. Demographic data was shown in Table 1. The distribution of age-specific NPC levels shows that the incidence of NPC increases with age group between 46 and 65 years to be the most common (20 patients, 66.7%). In sex group, male had the highest frequency distribution (86.7%), and the most common type of histopathology was a type of non-keratinizing squamous cell carcinoma in 23 patients (76.7%).

Table 1 Baseline Characteristic of NPC Patients

Characteristic of Patients	Nasopharyngeal Carcinoma	
	n	(%)
Age :		
≤ 25	2	6.7
26-45	6	20.0
46-65	20	66.7
> 66	2	6.7
Gender :		
Male	26	86.7
Female	4	13.3
Histopathology Type:		
Keratinizing squamous cell carcinoma	1	3.3
Non keratinizing squamous cell carcinoma	23	76.7
Undifferentiated carcinoma	6	1

Table 2 shows the examination results of bacterial colonization in NPC patients. Colonization was present in 27 patients (90%). Of these with positive swab culture, 3 patients had two types of bacteria namely *Staphylococcus epidermidis* and *Klebsiella pneumonia* in 1 patient, and *Staphylococcus aureus* and *Klebsiella pneumonia* each in 2 patients.

Culture and gram bacterial identification were performed and bacteria causing colonization in nasopharyngeal carcinoma patients was *Staphylococcus aureus* (36.36%). *Klebsiella pneumonia* (15.15%), followed by *Staphylococcus epidermidis* (9.09%) and *Klebsiella oxytoca* (9.09%), *Proteus mirabilis* (6.06%) and *Pseudomonas aeruginosa* (6.06%). Other species identified were *Staphylococcus non aureus* (3.03%), *Streptococcus a hemolysa* (3.03%), *Proteus vulgaris* (3.03%) (Table 3).

Table 2 Distribution of NPC Patients with Bacterial Colonization

NPC with Bacterial Colonization	Frequency	Percentage
	n	%
Colonized	27	90
Non-colonized	3	10
Total	30	100

Table 3 Bacterial Profile Based on Culture Examination

Bacterial Profile	N	%
<i>Staphylococcus epidermidis</i>	3	9.09
<i>Klebsiella pneumonia</i>	5	15.15
<i>Klebsiella oxytoca</i>	3	9.09
<i>Proteus mirabilis</i>	2	6.06
<i>Staphylococcus aureus</i>	12	36.36
<i>Staphylococcus bukan aureus</i>	1	3.03
<i>Streptococcus a hemolysa</i>	1	3.03
<i>Pseudomonas aeruginosa</i>	2	6.06
<i>Proteus vulgaris</i>	1	3.03
	30	100.00

Table 4 shows that the most bacterial colonization is at the age of ≥ 45 years with a total of 21 patients (77.85%), male sex was 23 patients (85.2%). nonsmokers were 25 patients (92.6%). histopathological type of non-keratin squamous cell carcinoma in 27 patients (100%). Based on bivariate analysis with Fisher exact test statistical tests showed that there was no significant correlation between age, gender, smoking and histopathology type as a risk factors and bacterial colonization in nasopharyngeal carcinoma patients with ($p > 0.05$).

Table 4 The Correlations Between Risk Factors and Bacterial Colonization

Risk Factors	Subjects with Bacterial Colonization (n=27)		Subjects with no Bacterial Colonization (n=3)		P
	n	%	n	%	
	Age				
≤ 45 tahun	6	22.2	1	33.3	1,000
≥ 45 tahun	21	77.8	2	66.7	
Gender					
Male	23	85.2	3	100	1,000
Female	4	14.8	0	0	
Smoking Risk					
Non-smoker	25	92.6	3	100	1,000
Smoker					

	2	7.4	0	0	
Histopatological Type					
Keratinizing squamous cell carcinoma					
Non keratinizing squamous cell carcinoma	0	0	1	33.3	0,1
	27	100	3	66.7	
Total	27	100	3	100	

4 Discussion

Bacterial colonization is one of the key factors in the process of respiratory tract infection. Nasopharyngeal colonization by potential respiratory pathogenic bacteria, such as gram-negative rod bacteria, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Moraxella catarrhalis* generally can cause clinical manifestations, and the presence of these potential respiratory pathogenic bacteria can be a source of transmission and spread.

In this study we found the highest frequency distribution of NPC was in the age group of 46 – 65 (66.7%). This finding is similar to several other studies which shows that incidence of NPC in the 40 – 60 age range.[8][9] [10]. The age distribution of patients diagnosed with NPC differs substantially depending on the disease endemicity. In areas with low incidence, NPC increases with age while in endemic areas, cases increase at age after 30 years, culminating at the age of 40 - 59 years, and decreasing thereafter. The process of NPC can be said that it will appear after latent infection which requires a long time, thus causing the number of sufferers to increase in adulthood [11].

Patients with NPC are more common in male than female as reported in almost all studies. This is assumed to be correlated with living and work habits that cause male to be more often exposed to carcinogens that cause NPC such as vapor exposure, dust, or chemicals in the workplace that can increase the risk of NPC 2 to 6 times higher in several studies [12]. Wei et al. in [13] have conducted research in China found 30,000 cases in male and 12,000 in female. Our study also found the similar result that the most common of NPC patients were in male.

Histopathology type of non-keratinizing squamous cell carcinoma is the main histopathology type in endemic countries such as China, Southeast Asia, and North Africa. Conversely, the histopathology type of keratinizing squamous cell carcinoma is more common in low-risk populations such as the white population of the United States. NPC non-keratinizing type of squamous cell carcinoma histopathology is closely related to Epstein-Barr virus infection [14]. Studies conducted at Adam Malik Hospital Medan found that non-keratinizing squamous cell carcinoma was the most common type of histopathology [9] [15]. Similar result was found in a study of 350 NPC patients at Hasan Sadikin Hospital Bandung between 2006 and 2010 [16]. Our study also found similar result showing the most histopathology type of NPC patients as non-keratinizing squamous cell carcinoma.

Cancer patients have a greater risk of getting serious infections even though cancer therapy and supportive care are now making significant progress [17]. Meanwhile, the bacteria that appear in certain tumours are often associated with the development of cancer where in many cases the bacteria that appear in the tumour can reflect local infections of existing malignant tissue [18]. Likewise patients with nasopharyngeal carcinoma can suffer from chronic colonization or lower respiratory tract infections. Currently, no studies have been found that identify colonization in patients with NPC.

Bacteria derived from the patient's normal microflora are the most common bacteria found in immunocompromised cancer patients. Microbiological findings show mostly gram-negative bacteria such as *Escherichia coli*, *P. aeruginosa*, and *Klebsiella pneumonia* species. While gram-positive bacteria usually found, among others, are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* and *Streptococcus mitis*. *Staphylococcus aureus* itself is a frequent cause of disease in communities and hospitals, between 20% and 41% of the human population has been found to be colonized by persistent *Staphylococcus aureus*, while others *Staphylococcus* sp. function as intermittent carriers of pathogens [19]. Our study found that *Staphylococcus aureus* were bacteria that grow in the most NPC patients followed by *Klebsiella pneumonia*, *Staphylococcus epidermidis* and *Klebsiella oxytoca* respectively.

In this study, we found there were no significant correlation between age group and bacterial colonization of NPC patients at Adam Malik Hospital and University of Sumatera Utara Hospital. However, in contrast to other study [20], the rate of colonization decreases with increasing age, i.e young adults aged 18-39 have a higher rate of colonization of *Staphylococcus aureus* compared to 59 years or more. Hikmawati also found that gram-negative bacterial colonization was more common in older patients (45-70 years) than in children [21]. This differences might be due to the differences in the number of samples.

We also found that there were no significant association between gender and bacterial colonization of NPC patients. This result is in line with other study where gender has no influence on the colonization of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* [22]. However, in contrast to study by Herwaldt, male gender had a higher risk to colonization by *Staphylococcus aureus* bacteria [23]. Similar results were also found for *P. aeruginosa* colonization in hospitalized patients where men had a higher risk [24].

There were also no significant correlation between smoking and bacterial colonization of NPC patients. The effect of smoking on colonization of pathogenic bacteria and nasal colonization is not yet fully understood and seems to be controversial, but it is suspected that cigarette smoke can cause damage by causing inflammation of the nasopharyngeal mucosa and eventually increase susceptibility to viral or bacterial colonization [6] - [25].

We also showed no significant correlation between histopathology type and bacterial colonization of NPC patients. This might be due to the fact that the number of samples with positive colonization is too large in the non-keratinizing squamous cell carcinoma type compared to other types. No other literature has yet been reported on similar studies of the correlation of histopathological types with bacterial colonization.

5 Conclusion

The most bacterial colonization profile in the bacterial culture of patients with nasopharyngeal carcinoma was *Staphylococcus aureus* in 12 patients (36.36%). There were no significant correlation between age, gender, smoking and type of histopathology and bacterial colonization in NPC patients.

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