



Expressions of Nuclear Factor-kappa B and Peroxisome Proliferator-activated Receptor-Gamma Proportional with Clinical Staging of Nasopharyngeal Carcinoma

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Abstract

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BACKGROUND: Nasopharyngeal carcinoma (NPC) is a malignancy induced by the mutation of the transcription factors nuclear factor-kappa B (NF-kB) and peroxisome proliferator-activated receptor-gamma (PPAR-gamma). There was no known of the study about the association and targeted therapy of NF-kB and PPAR-gamma-induced NPC.

AIM: This study analyzed and compared the proportion of NF-kB and PPAR-gamma and its association with the clinical characteristic of various NPC patients.

METHODS: This was a cross-sectional study and conducted in Adam Malik General Hospital. The samples were paraffin block tissue obtained from 58 NPC patients and underwent immunohistochemistry staining for NF-kB or PPAR-gamma overexpression in March–November 2018. Determination of overexpression was based on the immunoreactive score. The association of NF-kB or PPAR-gamma overexpression with the clinical characteristics of the patients was analyzed using Fisher's exact test.

RESULTS: This study showed a significant increase of NF-kB and PPAR-gamma ($p < 0.05$). Male was found common than women (3.46:1) with non-keratinizing squamous cell carcinoma as the most common form of NPC (75.9%) and the 41–60 years old is the most common age (56.9%). Overexpression of NF-kB and PPAR-gamma was found mostly in T3-T4 (66.0%; 69.6%), N+ (92.5%; 91.3%), and clinical Stage IV (67.9%; 73.9%), respectively.

CONCLUSION: The number of samples overexpressed was proportional to the clinical stage of NPC. This study provides an insight into the relationship of NF-kB and PPAR-gamma to NPC, suggesting their role in the development of malignancy.

Introduction

Derived from the squamous epithelial cells, nasopharyngeal carcinoma (NPC) is classified as the malignancies of the head-and-neck region [1]. NPC is a rare disease in the world with clear geographic and racial distribution [2]. The total of NPC cases was 0.7% of the total malignancies in the world. According to GLOBOCAN 2012, the incidence of NPC in Indonesia based on the age-standardized rate was 8.3/100,000 in male and 3.0/100.000 in female [3].

The involvement of various transcription factors has been established in many studies, suggesting that the dysregulation of these proteins could lead to carcinogenesis. Dysregulation of nuclear factor-kappa B (NF-kB) which is caused by the latent infection of Epstein–Barr virus (EBV) is responsible

for carcinogenesis [4]. The EBV altered the physiology of the signaling pathway through its oncoprotein, the latent membrane protein-1 (LMP-1). LMP-1 activates NF-kB to protect the cells from apoptosis [5]. NF-kB overexpression inhibits apoptosis of cancer cells lead to cell proliferation and also is involved in the progression of the tumor by regulation of matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) [6]. Like NF-kB, peroxisome proliferator-activated receptor-gamma (PPAR-gamma) also controls the homeostasis of the inflammation process [7]. It can whether to induce apoptosis or induce differentiation [8]. Ligand damage of PPAR-gamma such as polyunsaturated fatty acids and eicosanoids leads to malignancies development [9], [10]. The expression of PPAR-gamma inhibits cell proliferation and growth of the tumor. However, it can induce proliferation in some cancer cells or activated epidermal growth factor receptor (EGFR) signaling [11].

NF- κ B and PPAR- γ have similar mechanisms involving inflammation and specifically different in tumor development. There is little research about the association of NF- κ B and PPAR- γ with NPC and also there was no known targeted therapy of NF- κ B and PPAR- γ in NPC [12]. We did the study to found the association of NF- κ B and PPAR- γ in NPC. It was hoped that the results could be the basic research which may lead to any further research on the treatment of NPC.

Methods

Patients and samples

A total of 58 block paraffin selected as the samples from the patients who meet the inclusion criteria in this cross-sectional study. The inclusion criteria were NPC patients who agree to be the participant in the study and were diagnosed with NPC based on history taking, physical examination, and histopathology examination in 2016–2017. The study was composed of 45 men and 13 women. The patients were diagnosed histopathologically with NPC in the year 2016–2017. The criteria exclusion was no other malignancies found in the patients.

The World Health Organization classification of the types of NPC is used in diagnosing the histopathology staining. The clinical cancer staging was based on the American Joint Committee on Cancer (AJCC) 7th categorization. This study has been approved by the Health Research Ethical Committee, Medical Faculty of Universitas Sumatera Utara/Adam Malik General Hospital NO. 329/TGL/KEPK FK USU-RSUP HAM/2018 and the methods did not contradict with the Declaration of Helsinki.

Immunohistochemistry staining

The antibody used for immunohistochemistry staining was Santa Cruz NF- κ B p65 antibody (F-6) and Santa Cruz PPAR- γ antibody (8D1H8H4). The blocks were cut into approximately 4 μ m thick, deparaffinized by xylol 1, 2, and 3 for 5 min, then rehydrate it. The tissue section was put inside the Dako Epitope Retrieval to retrieve the antigens, heated at 98°C for 15 min. Immediately insert the Pap Pen into the tris-buffered saline (TBS), peroxidase block is used afterward. The tissue was then washed in the TBS again for 5 min. Continue with the Normal Horse Serum blocking for 5 min and rewashed it in the TBS, proceed with the NF- κ B or PPAR- γ antibody incubation. Two pathologists would then analyze the results, reactivity determined by the area of cells bind and intensity of the color.

The overexpression means that there are more than 10% of cells with positive staining based on immunoreactive score [13], [14].

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences 23.0 version. The correlation of NF- κ B and PPAR- γ overexpression with the size of the tumor, lymph node enlargement, and cancer staging was analyzed by Fisher's exact test.

Results

In this study, there were 58 paraffin block of NPC patients. The paraffin block was belonged to the patients with characteristic distributed based on gender, age, and type of histopathology which is shown in Table 1.

Table 1: Expression of NF- κ B based on the tumor size, lymph node involvement, and cancer staging

Type	NF- κ B expression, n (%)		p
	Overexpression	Negative	
Tumor size (T)			
T1-T2	18 (34.0)	5 (100.0)	<0.05*
T3-T4	35 (66.0)	0	
Lymph node involvement (N)			
N0	4 (7.5)	3 (60.0)	<0.05*
N+	49 (92.5)	2 (40.0)	
Distant metastasis (M)			
M0	17 (32.1)	5 (100.0)	<0.05*
M1	36 (67.9)	0	
Cancer staging			
I-III	17 (32.1)	5 (100.0)	<0.05*
IV	36 (67.9)	0	

*Fisher's exact test.

There were 45 male patients (77.6%) and 13 female patients (22.4%) in this study. The majority of the patients were around the age of 41–60 (56.9%). Studies showed the late adult stage (60 years old or more) as the peak incidence of NPC. Non-keratinizing SCC has the highest prevalence among the other types of histopathology.

We used the AJCC staging system in determining the classification of cancer by the size and tumor spread and the relation with NF- κ B and PPAR- γ expression which shown in Tables 1

Table 2: Expression of PPAR- γ based on the tumor size, lymph node involvement, and cancer staging

Type	PPAR- γ expression, n (%)		p
	Overexpression	Negative	
Tumor size (T)			
T1-T2	14 (30.4)	9 (75.0)	<0.05*
T3-T4	32 (69.6)	3 (25.0)	
Lymph node involvement (N)			
N0	4 (8.7)	3 (25.0)	<0.05*
N+	42 (91.3)	9 (75.0)	
Distant metastasis (M)			
M0	12 (26.1)	10 (83.3)	<0.05*
M1	34 (73.9)	2 (16.7)	
Cancer staging			
I-III	12 (26.1)	10 (83.3)	<0.05*
IV	34 (73.9)	2 (16.7)	

*Fisher's exact test.

and 2. It showed that high the T, N, M, and cancer staging classification, the more the antigens are expressed. It is shown that the clinical Stage IV has a higher prevalence than Stages I–III. Still, the N1–NIII stage was the stage with the most overexpression. The higher the clinical stages, the higher the staining intensity. There was a significant association of NF-kB expression with tumor size, lymph node involvement, the presence of distant metastasis, and cancer staging ($p < 0.05$). Based on PPAR-gamma expression, there was a significant association of PPAR-gamma expression with tumor size, lymph node involvement, the presence of distant metastasis, and cancer staging ($p < 0.05$).

Discussion

NF-kB is a nuclear factor that binds to an enhancer element of immunoglobulin κ light chain gene in B cells [15]. NF-kB is persistently active in multiple tumors and occupies an important role in carcinogenesis. It is a transcriptional factor that induces cell proliferation, apoptosis, inflammatory, and immune system [16]. Proliferation is activating by NF-kB through the expression of Cyclin D1 and c-myc as an oncogene that induces cell proliferation. Other proliferation inducers are TNF, interleukin (IL)-8, and IL-1 β which are also the target of NF-kB [17]. PPAR-gamma, which is a member of the PPAR family, is activated by several known endogenous ligands emerging from the metabolism of arachnoid acid and linoleic acid [18]. The expression of PPAR-gamma inhibits cell proliferation and growth of the tumor. However, it can induce proliferation in some cancer cells or activated EGFR signaling [11]. The main goal of the study is to analyze the expression of NF-kappa B and PPAR-gamma expression and their association with the clinical characteristics of NPC. The overexpression of NF-kappa B and PPAR-gamma was found to be related to the increasing stages of NPC. It explained the likelihood of NF-kappa B and PPAR-gamma involvement in the progression of the NPC.

This study revealed the overexpression of NF-kB in 53 of 58 NPC patients and the overexpression of PPAR-gamma in 46 of 58 NPC patients. The overexpression of PPAR-gamma and NF-kB significantly correlates with tumor size, lymph node involvement, and distant metastasis and high cancer staging. NF-kB is found overexpression in NPC patients [19]. NF-kB overexpression inhibits apoptosis of cancer cells lead to cell proliferation. It is involved in the progression of the tumor by transcriptional regulation of invasion-related factors, such as MMP-9 and VEGF which related with advanced clinical stage of NPC [6], [20], [21]. Some studies revealed the role of PPAR-gamma-induced angiogenesis by enhancement VEGF in cancer cells.

Like angiogenesis, the role of PPAR-gamma was unclear whether as pro-proliferative or antiproliferative according to the tumor [22].

Several studies were done in detecting NF-kB overexpression in NPC. Zhang *et al.* found expression of NF-kB in 27 of 42 NPC patients. That study also showed that the expression of NF-kB was associated with poor outcome of the disease [19]. A study by Sun *et al.* showed expression of NF-kB in 57.6% of NPC tissue and resulted in significantly of NF-kB upregulation in the case. In their study, they did not found the significant association of NF-kB expression with patient age, gender, and metastatic stage. However, the study found that NF-kB was only associated with lymph node metastasis. According to the 5 years of survival rate, there were no significant differences between lower NF-kB and higher NF-kB in NPC patients [23]. Shi *et al.* in their study revealed the increased level of NF-kB and its transcriptional cofactor RELB and BCL3 in the primary tumors of NPC [24]. Those studies revealed the involvement of NF-kB in NPC.

However, the relation of PPAR-gamma overexpression with NPC remains unclear. A study by Wright *et al.* showed that PPAR-gamma expression in 34 of 44 from specimens of head-and-neck cancer with oral and oropharyngeal cancer had a lower expression [25]. Other cancer that had been known to be related with PPAR-gamma is prostate cancer. Several studies showed that there was a significant correlation between PPAR-gamma expression and the staging of prostate cancer. PPAR-gamma expression in the disease was related to an increase in the stage and grade of prostate cancer [26]. Despite limited studies that have been conducted on the PPARs, they have been linked to various conditions such as the levels of lipoprotein, cardiovascular disease, diabetes, obesity, and malignancies [27]. PPARs exposed an active role in glucose and lipid metabolism, and the regular functioning of the adipose cells [28].

Conclusion

This study showed a significant increase of NF-kB and PPAR-gamma as the basic data for the role of those two in the development of NPC. A further study is needed to fully understand the mechanism of the two transcription factors in causing cancer. The clinical impact of the study is as basic research for further study to find a better understanding of the use of NF-kB and PPAR-gamma as a prognostic factor and targeting therapy study in NPC patients. More numbers of specimens in which about more than 1000 samples are needed to determine any correlations of the two with the best precision, working to alter the mutations of genes.

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