

Association of Protein Expression p53 Mutants with Regional Lymph Gland Status on type III Carcinoma Nasofaring Patients

Riza Aswarin¹ · Muhtarum Yusuf¹ · M. S. Wiyadi¹

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Abstract Nasopharyngeal carcinoma (NPC) is a malignant disease originating from the nasopharyngeal epithelial cells. The molecular mechanism of cancer occurrence is a change in the oncogene and tumor suppressor genes. One of the tumor suppressor genes that mutate in cancer cells is the mutant p53 gene. One of nasopharyngeal carcinoma progression is determined by the status of regional lymph gland. The enormous regional lymph node has a poor prognosis. To analyze the expression of the mutant p53 protein in Nasopharyngeal carcinoma (NPC) that correlated with regional lymph gland status (N) as a clinical manifestation. Expression of mutant p53 protein from NPC tissue paraffin block with immunohistochemical staining technique was using monoclonal rabbit Anti Human p53 clone 318-6-11 (Dako, North America, Inc., 6392 Via Real Carpinteria, CA 93013 USA), microscope light binoculars was assessed visually by an Anatomical Pathology Consultant. Positive expression of p53 mutants was obtained 57.58% from all the sample in N0 by 0 subjects, N1 was 6 subjects, N2 was 7 subjects, and N3 was 7 subjects. The results of Mann–Whitney *U* test was $p = 0.706$, then there was no significant ($p > 0.05$) correlation between positive expression of p53 protein in type III WHO NPC with the regional lymph gland were N0, N1, N2, and N3. There was no significant between expression protein p53 mutants' regional and lymph gland in type III WHO NPC.

Keywords Nasopharyngeal carcinoma · Protein p53 mutants · Lymph mutants

Introduction

Nasopharyngeal carcinoma (NPC) is a type of squamous epithelial cancer that originates from the surface of the lateral nasopharyngeal wall. In South China, Taiwan, and Southeast Asia, especially Indonesia NPC is the most common malignant head and neck tumor. The prevalence rate in Indonesia based on histopathology is reported to be about 6.2 cases for each 100,000 population every year [1]. In Surabaya, based on data from the oncology division department of Otorhinolaryngology, Dr. Soetomo General Hospital in 2000–2002, NPC was the most malignant tumor in the head-neck. The number of NPC during the year was 855 cases [2].

The development of molecular biology techniques lately is one of the causes that carcinogenesis is the failure of tumor suppressor gene p53, In the expression of a normal cell of p53 protein is low. When DNA was damaged, the p53 protein will automatically activate also activated p21 that called CDK inhibitor. p21 will bind and activate the CDK complex which will cause Rb phosphorylation to be inhibited and release of transcription factor stalled so that cell cycle stops at stage G1-S. When the cell cycle stops, the DNA has a chance to repair itself before entering the next division stage. If the DNA damage is severe and unrepaired then the cell will enter the path of apoptosis. The p53 (wild-type) protein gene has mutations in 50% of all cancers in various human organs and considered the most mutated gene, including breast, colon and lung cancers. One of the causes of NPC factors is the inactivation of the p53 protein gene that leads the formation of the mutant

✉ Muhtarum Yusuf
yusufmuhtarum@gmail.com

¹ Department of Otorhinolaryngology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Jalan Prof. Dr. Moestopo No. 47, Surabaya 60131, Indonesia

p53 protein (Nul type), thus the normal function of the p53 protein is lost. This loss of function causes an increase in apoptotic inhibiting genes that resulting in decreased apoptosis and the cell becomes more resistant to radiotherapy [3, 4].

Various studies have attempted to determine the prognostic factors of high-risk NPC patients. Kind of histopathology NPC is known as one of the factors that determine prognosis, NPC type undifferentiated carcinoma (WHO type III) has a higher survival rate compared to type II and type I WHO NPC. Then, TNM staging is also one of the prognostic factors in NPC, where the patients with a higher staging usually have a poorer prognosis. It is said that 16% of NPC patients with regional KGB negative (N0) found metastases long after radiotherapy [5, 6].

Based on previous studies it was found that NPC happened due to the proliferation of p53 epithelial cell protein clones that begins with EBV infection and lead to p53 protein mutations [7]. This mutation leads to loss of function of the normal p53 protein (wild type) in inhibiting growth and cell differentiation which then continues in nasopharyngeal malignancies. Research in Medan concluded that the expression of protein p53 mutants in NPC was 76.5% [8]. A study in Thailand 53 paraffin blocks of NPC were 42 (79%) that showed mutant p53 protein expression [9]. While, in Korea it was found that 2/3 (71.1%) of 38 NPC cases occurred an expression on p53 protein especially in type III WHO [8–11].

Based on the role of suppressor gene of mutant p53 protein tumor as one of the factors that causing NPC and correlation between protein p53 mutant and regional lymph gland is not clear, then, the authors feel the need to conduct research, so it is expected to be useful to estimate tumor response to therapy in NPC patients. Up till now, the research has never been done in the Department of Otorhinolaryngology, Faculty Medicine of Universitas Airlangga/Dr.Soetomo General Hospital Surabaya.

Methods

The inclusions sample as enrolled in this study were: type III NPC WHO patient that has N0, N1, N2, and N3 lymph enlargement; paraffin blocks of nasopharyngeal tumor biopsy that have adequate tumor tissue for immunohistochemical examination (4 microns thick). While, the exclusion criteria were: patients who have already received definitive therapy i.e., radiotherapy, chemotherapy or a combination of both.

The procedures were checking the status of type III NPC WHO patients lymph glands and collecting a large number of samples for each group N0, N1, N2 and N3. Searched for paraffin block of NPC tissue's each subject for

Immunohistochemical immunohistochemistry with rabbit monoclonal human antibody p53 Clone 318-6-11 (Dako North America, Inc., 6392 Via Real Carpinteria, CA 93013 USA) that performed an assessment of protein p53 mutant expression by anatomical pathology physician as a consultant.

Results

The highest number of NPC patients in the age group 40–49 years old were 17 cases (51.52%), followed by the 30–39 and 50–59 years group was 7 cases (21.21%) respectively (Table 1). The youngest age was 30 years old and the oldest was 71 years old. Most of the sexes were male with 21 cases (63.64%) and a female was 12 cases (36.36%), then, the comparison between men and women was 2: 1 (Table 2). Ethnicity data on NPC sufferer is Javanese by 28 patients (84.85%), Madura Ethnic was 4 patients (12.12%) and Dayak Ethnic was 1 patient (3.03%) (Table 3) while most of the patients job were farmers by 13 patients (39.39%) followed by self-employed 11 patients (33.33%) (Table 4).

The results of protein p53 mutant examination in NPC with N0 regional lymph gland status were negative on 3 samples, In N1 was 6 samples positive result and 1 sample of the negative, In N2 was 7 samples positive result and 3 negative result samples, whereas in N3 was 6 samples positive result and 7 samples with the negative result. Then, positive mutant p53 expression of 57.58% of all samples, and negative mutant p53 expression of 42.42% of all samples at N0, N1, N2, and N3. Mann–Whitney *U* test results obtained $p = 0.706$, these data suggest that there was no significant correlation between mutant p53 expression and N0, N1, N2, and N3 regional lymph glands (Table 5).

Immunohistochemical examination results in NPC tissue identified by the presence of dark brown color in cell membranes and tumor cell cytoplasm. Observation and analysis of mutant p53 protein expression were performed with a binocular microscope with 400× magnification (Fig. 1).

Discussion

Previous research in Turkey obtained the expression of the p53 mutant protein was 85.5% of 97 NPC patients as samples that performed immunohistochemical examination while the remaining 14.5% negative. There was no significant correlation between expression of protein p53 mutant and histology type, stage, age and sex [11]. A study in Tunisia reported that 81% of 69 samples of positive

Table 1 Age distribution

Age (y/o)	N	%
30–39	7	21.21
40–49	17	51.52
50–59	7	21.21
≥ 60	2	6.06
Total	33	100.00

Table 2 Sex distribution

Sex	N	%
Male	21	63.64
Female	12	36.36
Total	33	100.00

Table 3 Ethnic's distribution

Ethnic	N	%
Javanese	28	84.85
Madura	4	12.12
Dayak	1	3.03
Total	33	100.00

Table 4 Jobs distribution

Jobs	N	%
Farmer	13	39.39
Company employee	3	9.10
Self-own	11	33.33
Unemployee	6	18.18
Total	33	100.00

Table 5 The results of protein expression p53 mutants based on lymph gland region status (N0, N1, N2, N3)

Score	N0	N1	N2	N3	%	<i>p</i>
–	3	1	3	7	42.42	0.706*
+	0	6	7	6	57.58	
Total	3	7	10	13	100.00	

**p* > 0.05 (no significant)

biopsy NPC patients that over 30 years old with immunohistochemical p53 mutant proteins, in this case, the presence of tumor and virus interactions have its own

characteristics and the presence of more EBV virus genome also anti VCA levels, EBV IgA and anti EA EBV high. While, in Thailand, 79% of 53 cases and in Medan was 76.5% of 34 samples. This contributes to the avoidance of apoptotic processes [12]. From the results, it indicates that the expression of protein p53 mutants in NPC ranges was between 50–80% [8, 9].

Other results of the statistical tests showed that there was no association between mutant p53 expression of metastatic nodules in NPC. According to this study, the role of mutant p53 protein expression has started early on tumor development, before the occurrence of spread to the lymph glands and distant metastases. Tumors that have occurred a far metastasis, the expression of protein p53 mutant role is small. Proteins that play a role in cell cycle control were gene suppressor and oncogene tumors. The role of p53 protein in the regulation of cell cycle was to inhibit cell division, where the p53 protein will trigger the transcription process of p21. The increased of p21 will cause the p53 protein to inhibit all CDKs, While, non-functioning of CDK will impacts the cyclin to not form a complex with CDK, this affecting in a stop cell cycle. To re-trigger the cell division cycle the contributing factor was the MDM-2 protein. The activity of this protein will suppress the activity of the p53 protein. The low activity of p53 protein resulted in decreased expression of p21 as a result of CDK did not experience inhibition, so a cycling will form a complex with CDK. The complex bond between CDK-cyclin will cause the cell cycle to keep continue. If there was a mutation of p53 protein then the produced of it was inactive and not able to trigger the formation of p21. The low expression of p21 results in the CDK uninhibited and the cell division cycle continues. On the other hand, the mutation of the p53 protein results in the disruption of protein BAX activity, thus Pt-pore in the mitochondrial membrane unable to open and eventually the cells were not apoptotic.

Accordingly, if there was a mutation in the Bcl-2 gene, then Bcl-2 protein will be overactive and result in suppression of BAX protein. This emphasis was caused Pt-pore to become more difficult to open and eventually the cells become immortal. The p53 protein in addition to working to suppress CDK activity also triggers an increase in the activity of the BAX protein. The BAX protein suppresses Bcl-2 activity in the mitochondrial membrane, resulting in decreased Bcl-2 function resulting in Cytochrome-C release to the cytosol. Cytochrome-C then activates Apaf-1. Furthermore, Apaf-1 activates caspase Kaskade and apoptosis occurs [13–15]. Various examination methods or techniques used to detect the expression of mutant p53 proteins such as cytogenetics, fluorescent in situ hybridization (FISH), polymerase chain reaction

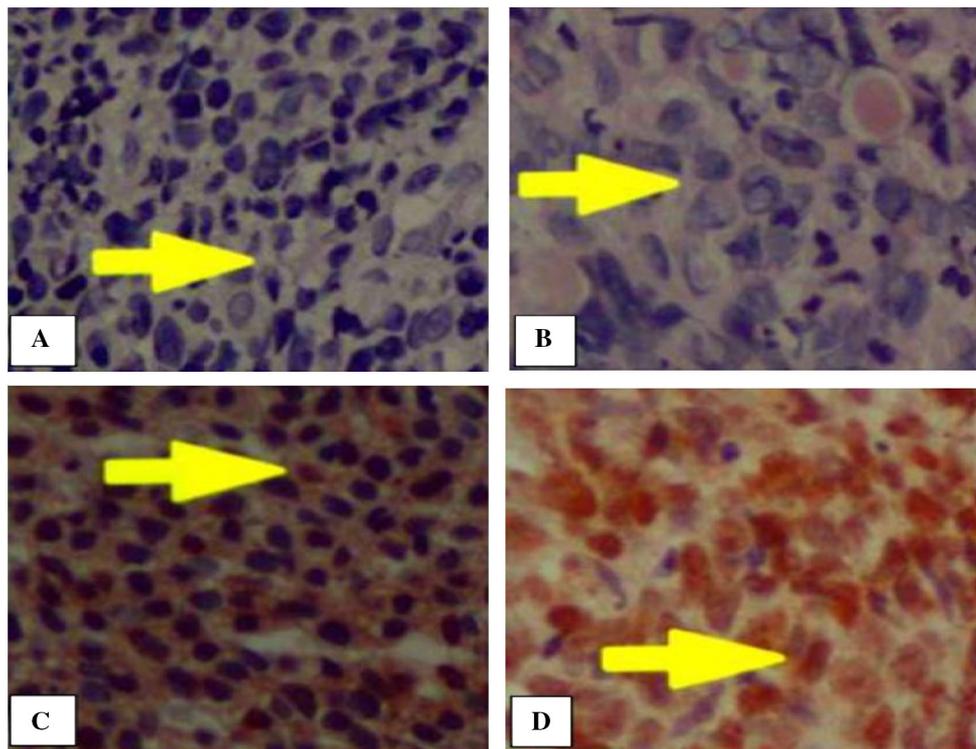


Fig. 1 The result of the mutant p53 protein painting on KNF tissue with immunohistochemical technique, the positive expression on cytoplasmic tumor cells in brown color; **a** P53 mutant negative expression, **b** mutant p53 expression < 10%, **c** expression of mutant

p53 10–15%, **d** expression of mutant p53 > 50%. Negative expression was not found in brown cytoplasm in tumor cells; the yellow arrows shows brown in the cytoplasm and tumor cell membrane (400× enlargement)

(PCR), DNA sequencing single strand conformation polymorphism (SSCP) and microarrays [16].

The results could influence by various factors such as the method used, the characteristics of the research population like tumor histopathology, staging, ethnicity, gender, smoking history and geographic conditions. The way of storage the dosage preparations could decrease the immunoreactivity of paraffin since the research tissue was usually taken long ago and stored for later use. In another study that comparing biopsy preparations and cell cultures of p53 proteins and MIB1 antigens stored at 20 °C for 16 weeks with newly taken tissue, p53 expression was significantly decreased. Furthermore, this study showed that at different storage temperatures, the different p53 expression was obtained. The duration of fixation in formalin also decreases epitope immunoreactivity [17].

Immunohistochemical examination was the primary tumor from nasopharyngeal biopsy results that associated with the status of the regional lymph glands of the patient, thus the results obtained were less than optimal, it might be different if the examined regional lymph glands were associated with the results of the biopsy from the primary tumor in the nasopharynx. The importance of distinguishing the measurement method used was to detect the mutant p53 protein expression analysis, the continued of research

correlation, the characteristics of each patient population, and different molecular markers that could be inferred as the cause of mutation.

Conclusion

There was no correlation between mutant p53 protein expression and regional lymph glands status in type III WHO NPC patients. A further study is needed which involves other variables such as protein p21 to determine the correlation of mutant p53 protein expression in the regional lymph glands to the magnitude of primary tumors in the nasopharynx.

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