p53 protein overexpression in nasopharyngeal carcinoma in Indonesian patients

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Abstrak

p53, baik gen maupun proteinnya, merupakan salah satu unsur genetik yang paling banyak diteliti pada penyakit kanker. Oleh karena prevalensi Karsinoma nasofaring (KNF) hanya tinggi pada daerah tertentu saja, terutama di beberapa negara Asia, maka tidak banyak dijumpai tulisan mengenai ekspresi p53 pada KNF dalam kepustakaan internasional. Tulisan ini membahas mengenai ekspresi protein p53 dengan pewarnaan imunohistokimia pada 48 pasien KNF Indonesia dan menghubungkannya dengan 2 klasifikasi KNF yaitu menurut WHO dan FORMULASI KERJA. Ekspresi protein p53 ditemukan positif pada karsinoma sel skuamosa, karsinoma tipe A dan karsinoma tipe B berturut turut sebanyak 100%, 82,6% dan 62,5%. Positivitas ekspresi p53 ini berhubungan bermakna dengan klasifikasi FORMULASI KERJA tersebut, sehingga memberikan kesan adanya makna prognostik p53 pada KNF.

Abstract

p53 gene and its protein is one of the most widely studied genetic abnormalities in cancer. However due to the restriction of nasopharyngeal carcinoma (NPC) to certain areas, mostly in Asia, scant attention has been paid to the over-expression of p53 in the high prevalence of NPC in the international literature. This study examined the immunohistological expression of p53 in 48 Indonesian NPC patients and correlated it with two NPC histological classifications. p53 protein over-expression were respectively found in 100%, 82.6%, and 62.5% of squamous cell carcinoma, type A carcinoma and type B carcinoma of Working Formulation classification. This statistically significant correlation gives the impression that p53 may have prognostic relevance in NPC.

***Keywords**: p53 protein, nasopharyngeal carcinoma, immunolabelling, grading*

INTRODUCTION

p53, a 373 amino acid nuclear phosphoprotein was first identified in 1979.[1] Although initially thought to be an oncogene, it has since been recognized that p53 functions as a tumor suppressor gene.[2] Numerous subsequent reports have shown that p53 abnormalities occur frequently in a very wide range of tumors. Indeed, abnormalities of p53 appear to be the most common genetic change in human cancer.[3]

Nasopharyngeal carcinoma (NPC) is highly prevalent in several Asian countries and is rare in most Western countries. Because of this difference in prevalence, scant attention has been paid to the over-expression of p53 in NPC, particularly its correlation with histological subtypes.

Based on pathology-based cancer registry statistics, NPC, mostly of the undifferentiated type, was ranked as the fourth most common neoplasm in Indonesia (Department of Health, Indonesian Association of Pathologists, Indonesian Cancer Society. Cancer in Indonesia 1994. Unpublished histopathologic data). This study examined the over-expression of p53 in 48 cases Indonesian NPC patients, employing an immunohistochemical method.

MATERIALS AND METHODS

The biopsy specimens from 48 NPC cases at the Department of Anatomic Pathology, Faculty of Medicine, University of Indonesia, Jakarta, were examined. Paraffin-embedded specimens were sent to the Institute of Medical and Veterinary Science, Adelaide, Australia (where A S-Y L worked at the time of this research) for immunolabelling. The

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sections were subjected to microwave epitope retrieval as previously described.[4] Briefly, deparaffinised and rehydrated sections were immersed in 10 mM citrate buffer at pH 6.0, in a closed container, and subjected to microwave irradiation in a domestic microwave oven with a carousel (NEC Model 702, 650 watt) at maximum setting. Five minutes after boiling point was attained, the buffer was maintained at simmering temperature for a further 10 minutes after which the power was switched off. The sections allowed to remain in the hot buffer for another 20 minutes before removal for immunostaining. A standard streptavidin peroxidase technique was employed with anti-p53 monoclonal antibody D07 (Dako, Sydney, Australia) at 1:100 dilution; enzyme digestion was not employed.

The extent of nuclear staining for p53 protein was scored on a scale of 0-4+ where: 0 represent no staining; 1+ = <10% of tumor cells stained; 2+ = 10-25% of tumor cells stained; 3+ = 26-50% of tumor cells stained; and 4+ if more than 50% of tumor cells were stained.

Representative hematoxylin and eosin-stained tumor sections were examined and classified according to the WHO classification[5] into squamous cell carcinoma (SCC), differentiated nonkeratinizing carcinoma (NKC) and undifferentiated carcinoma (UC). In addition, a classification similar to that proposed by Hsu et al,[6] and employed in Indonesia[7] was used. This 'Working Formulation' classification divides NPC into three subtypes (Table 1).

Table 1. Working formulation classification

Types / features	Keratinizing SCC	Type A carcinoma	Type B carcinoma
Histologic pattern	Flat pavemented	Syncytial	Syncytial
Tumor cells	Intercellular bridges and/or keratinization	Large, owl-eye like nuclei	Smaller, more basophilic nuclei
		Hyperchromatic, spindle cells	Spindle cell, fine chromatin
Pleomorphism		Evident	Little
Malignancy	High-grade	Intermediate	Low-grade
5-year surv.rate	21%	30-40%	60-70%

RESULTS

Positive nuclear staining for p53 protein was found in 38 (79%) of the 48 cases, the distribution shown in Table 1. Sixteen (33%) tumors revealed 1+ staining and another eight (17%) showed 2+ staining. Eleven (23%) tumors showed 3+ staining and only 3 (6%) showed 4+ staining.

The distribution of p53 over-expression in NPC typed according to the WHO classification is shown in Table 2. Positivity was observed in all nine cases of squamous cell carcinoma although only six showed more than 3+ positivity in tumor cells. The two cases of differentiated non-keratinizing carcinoma were positive but only as 2+ or less; on the other hand, the undifferentiated carcinoma group showed a variable degree of p53 staining, with eight cases showing more than 2+ cell positivity, six cases 2+ positivity, and 13 cases less than 1+ positivity. The remaining 10 cases did not stain at all for p53.

Table 2. p53 immunostaining in Nasopharyngeal Carcinoma

Percentage of positive tumor cells	Number of cells (%)
0	10 (21)
1+	16 (33)
2+	8 (17)
3+	11 (23)
4+	3 (6)
Total	48 (100)

Over expression of p53 protein appeared to show correlation with the 'Working Formulation' classification as proposed by Hsu et al[6] (p=0.03). There was staining in 100% of the nine cases of keratinizing squamous cell carcinoma, 82.6% (19 out of 23 cases) of Type A carcinoma and 62.5% (10 out of 16 cases) of Type B carcinoma (Table 3).

Table 3. p53 immunostaining in subtypes of NPC according to the WHO classification

p53 immunostaining	Histological Subtypes (WHO classification)		
	SCC	NKC	UC
0	0	0	10
1+	2	1	13
2+	1	1	6
3+	5	0	6
4+	1	0	2
Total	9	2	37
% Positive	100	100	72,9

DISCUSSION

The human p53 gene is located at chromosome 17p13.1 and encodes a 373 amino acid nuclear phosphoprotein, which is involved in the regulation of cell proliferation.[8,10] Studies in human cancers have revealed that p53 gene is the most frequently affected gene in a wide range of tumors, including cancers of the colon, lung, esophagus, breast, liver, brain, and hematolymphoid malignancies.[11,13]

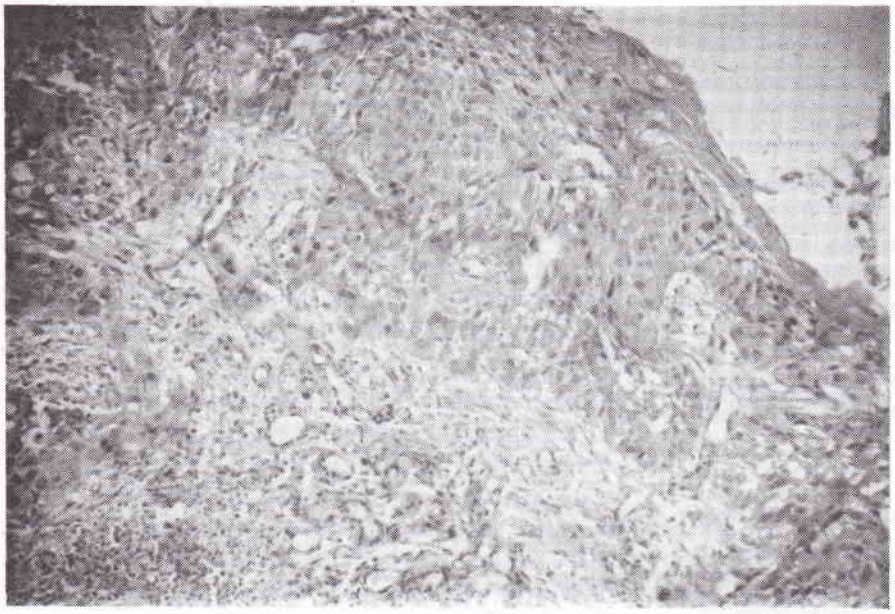
Chromosomal analysis in several established NPC cell lines have shown multiple genetic aberrations, including chromosome 17p13.

There are several techniques available for the detection of p53. Detection at the chromosome and gene level can be performed by Southern blotting, in situ hybridization or PCR; whereas, identification of the protein can be done with immunohistochemical techniques which show good correlation with p53 mutation.[14,16] The monoclonal antibody D07 employed in this study detects both mutant and wild type p53. Wild type p53 has a short half life of less than 30 minutes and is located in the nucleus. Mutant p53, on the other hand, has a prolonged half life of several hours, rendering it readily detectable by immunolabelling method. It has been demonstrated that p53 protein may bind to cellular proteins such as the mdm2 oncogene product and heat shock protein 70, as well as to several DNA viral proteins including E6 HPV16 protein,[17,18] SV40T antigen, and E1b protein from adenovirus type 5, all leading to its functional inactivation and stabilization.[19] While the presence of immunostained p53 protein does not necessarily indicate a gene mutation, the over-expression detected by immunostaining may be a useful indicator of altered wild type p53 function resulting from inactivation and stabilization through one of the preceding mechanisms. It is also recognized that various other cellular insults can induce wild type p53 expression in tumor cells. Within a population of cells in-vivo there is different sensitivity to genotoxic stimuli, yet to be identified, which can stabilize wild type p53, leading to elevated protein levels.[20] Study by Leung et al[21] in EBV-associated gastric carcinoma and head-and neck carcinoma showed a weak to moderate p53 expression, while there was a statistically significant difference of p53 expression between EBV-associated gastric carcinoma and EBV-negative gastric carcinoma. This suggested a non-mutational mechanism of p53 upregulation. Murono et al[22] have investigated the association of EBV with status of p53 protein expression in NPC, examined the expression of EBV gene and gene product, p53 protein and bcl-2 protein. Significant correlation was found between the expression of EBV and p53 protein, but not between p53 protein and LMP1. They suggested that some EBV-encoded protein may play a role for nuclear accumulation of p53 protein. Interference by EBV infection may thus cause altered p53 function through epigenetic influence.

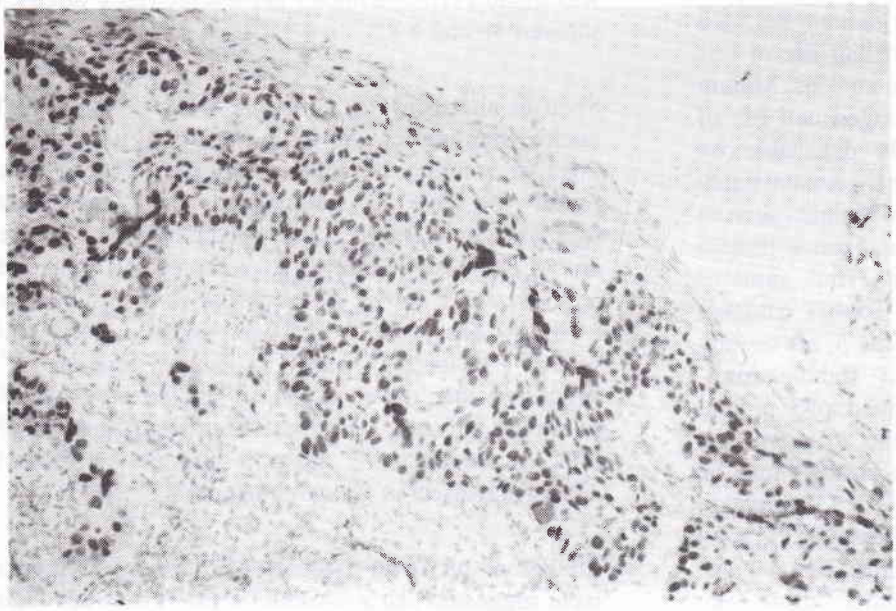
There have been few previous reports examining the over-expression of p53 protein in NPC using immunohistochemical methods. Niedobitek et al[23] detected p53 over-expression in five of nine EBV-negative squamous cell NPCs and in nine of thirteen EBV-positive cases of undifferentiated carcinoma. In a study of 41 cases from Hong Kong, Porter et al[24] found p53 over-expression in 70% of cases, 12% showing strong immunostaining for the protein. Our study showed similar result, and strong positivity was found even in a higher percentage (29% of cases showed 3+ and 4 +).

Sheu et al[25] using the antibody D07, demonstrated nuclear staining for p53 in 96 (95%) of 101 lesions. Positive staining in adjacent dysplastic cells was found in 79% of carcinomas with associated dysplastic epithelium. Based on their findings, these authors suggested that p53 over-expression occurs at an early stage in the development of NPC. Further more, by observing the co-expression of bcl-2 and p53 in 77% of NPC cases in Taiwan, Sheu et al[26] suggested that mutated p53 or altered function of wild-type p53 may contribute to the pathogenesis of NPC, in which bcl-2 and p53 may play a crucial synergistic effect in the carcinogenesis of NPC.

Studies of p53 over-expression in breast carcinoma have suggested that a correlation exists between high levels of expression and advanced stage of disease and metatastic tumor spread.[27] However, elevated levels of p53 have not been shown to be of prognostic relevance in other epithelial tumors such as small cell carcinoma and adenocarcinoma of the lung, and colonic carcinoma. None of the three immunohistochemical studies in NPC found a correlation between p53 over-expression with survival, potential for metastasis, clinical stage, or histological parameters including grade and lymphocytic infiltration.[23,25]

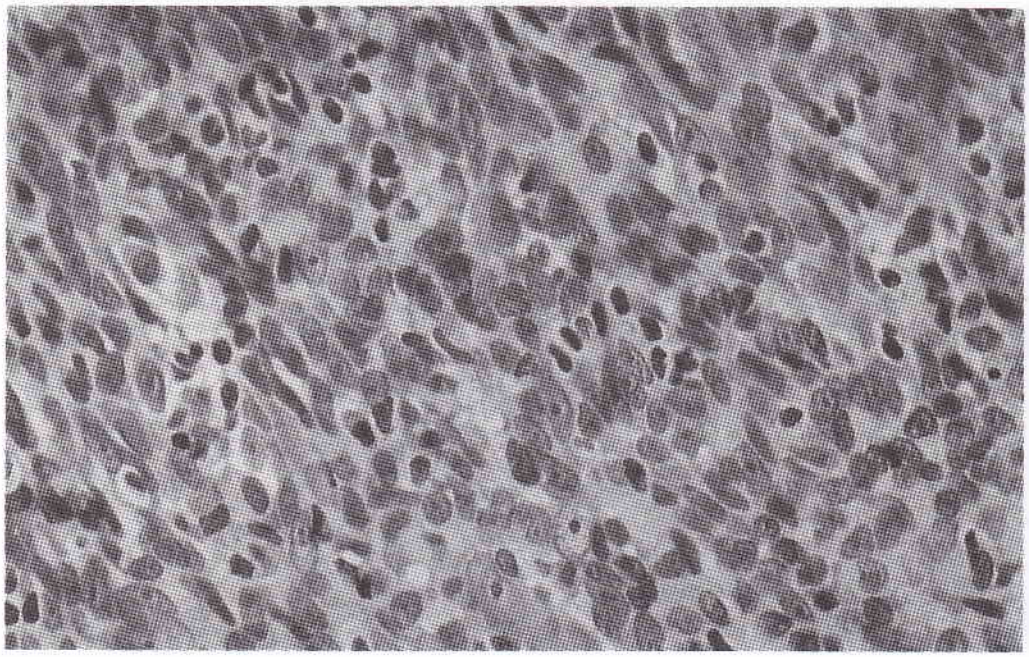


(a)

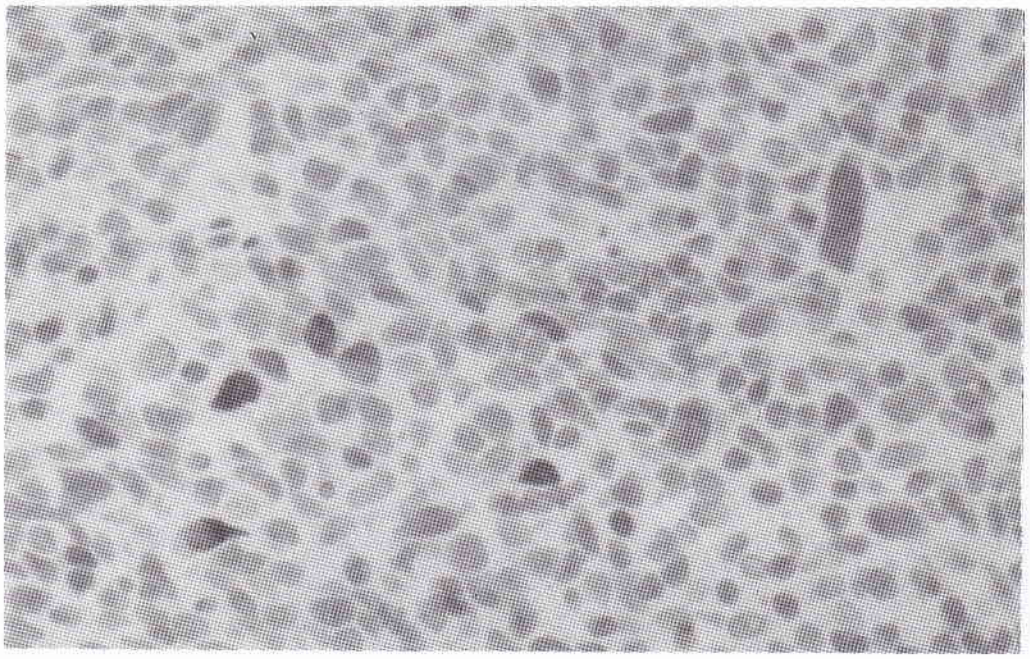


(b)

Figure 1. (a) Keratinizing squamous-cell carcinoma (H&E) showing 4+ positivity for p53 protein (b)



(a)



(b)

Figure 2. (a) Type A carcinoma (moderate grade malignancy) (H&E) showing 4+ positivity for p53 iimunostaining (b) The pleomorphic tumor cells display prominent nucleoli and spindled forms

Our findings of 79% positivity in 48 cases of NPC, of which 46% stained more than 10% of tumor cells, is in concordance with these previous reports. We did not find a correlation of p53 over-expression with subtypes of NPC when classified according to the WHO system. However, this was not unexpected as the WHO classification is not related primarily to tumor grade, but rather to the morphology of the tumor cells. On the other hand, the Working Formulation as proposed by Hsu et al[6] has been proven to be of prognostic correlation. Employing the alternative Working Formulation on which the Indonesian classification is based,[7] we found that there was a statistically significant correlation in this study. All cases of keratinizing squamous cell carcinoma (high grade malignancy) stained for p53, while positivity in Type A carcinoma (intermediate grade malignancy) was 82.6% and in Type B carcinoma (low grade malignancy) was 62.5%. It is of interest that these three subtypes of NPC showed significant different rates of survival,[7] therefore suggesting that p53 may have prognostic relevance in NPC.

Masuda et al[28] who made a clinicopathologic study correlating p53, bcl-2 ,Ki-67, sensitivity to radiation, incidence of distant metastases and survival, reported that NPC patients who were positive for p53 tended to be resistant to radiotherapy and to have significantly poorer prognosis. They concluded that the enhanced expression of p53 may be a prognostic factor in NPC patients whose tumor is resistant to DNA-damaging therapy.

The role of p53 as an independent prognostic parameter should be tested through multivariate analysis in conjunction with other prognostic variables and further investigations are necessary to identify appropriate cut-off values of p53 positivity as suggested by Dowell and Hall.[15] Furthermore, it may be necessary to examine the relevance of not only the percentage of positive staining tumor cells but also to employ some quantitative method which incorporates the intensity of immunostaining such as can be performed with image analysis.

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