P53 AND Ki67 BIOMARKERS AS PROGNOSTIC FACTORS OF NON SMALL CELL LUNG CARCINOMA

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Abstract- Biomolecular behavior of tumor cells has become attractive to investigators. p53 as an oncosuppresor gene has been core of various studies and Ki67 is a nuclear protein involved in proliferation process. Stopping of onco-suppressor function theoretically allows proliferative system to get out of any control. In this case-control study we evaluated 50 patients with non small cell lung cancer, considering the association between P53 oncoprotein and Ki67 with rate of tumoral cells differentiation. We found the concurrent move of P53 and Ki67 according to the rate of differentiation and a significant risk (odds ratio) for being poorly differentiated in samples having higher rates of these two factors. We suppose that mutant P53 protein not only may be used as an objective finding for tumor grading, but probably as a practical point of approach for determining prognosis and planning therapy for this patients.

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INTRODUCTION

Non small cell lung carcinoma (NSCLC) has been studied epidemiologically and etiologically and from many other aspects during the past decade. One of the most important influencing factors for predicting the outcome of patient is biological behavior of neoplasm (1-6).

Apart from conventional stage and grading systems, the oncogenes and proliferation factors at biomolecular levels have critical importance for management of the patient and biological classification of tumors (3, 7-10). Mutation of p53 gene is known as a proto-oncogenic state of most cancers as well as NSCLC (1, 7, 14). We know that as its oncosuppresor control effect is removed, the cell will be allowed to accept more and more mutations, above DNA repair system capacity, and this gets rise to poor differentiation. On the other hand, cell's proliferation system will act autonomically out of control. So theoretically, the poorer differentiation grade, the higher proliferation rate. Ki67 is a cell cycle associated antigen of nuclear matrix which express in all cell growth phases except resting phase (G0) (15). Number of cells stained positively by monoclonal Ki67 antibody correlates with tissue proliferation rate and grade (15, 16). This study was designed to check this concept and also the relation between the Ki67 and P53 expression.

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MATERIALS AND METHODS

In this case-control study 50 patients from two medical center, Shariati and Rassoul Akram Hospitals, diagnosed as NSCLC from 1999 to 2003, seen by two separate pathologist were included and divided into two group of high grade tumor (as cases) and low grade tumor (as controls).

Case group included 19 patients with high grade NSCLC and control group included 31 patients with low grade NSCLC.

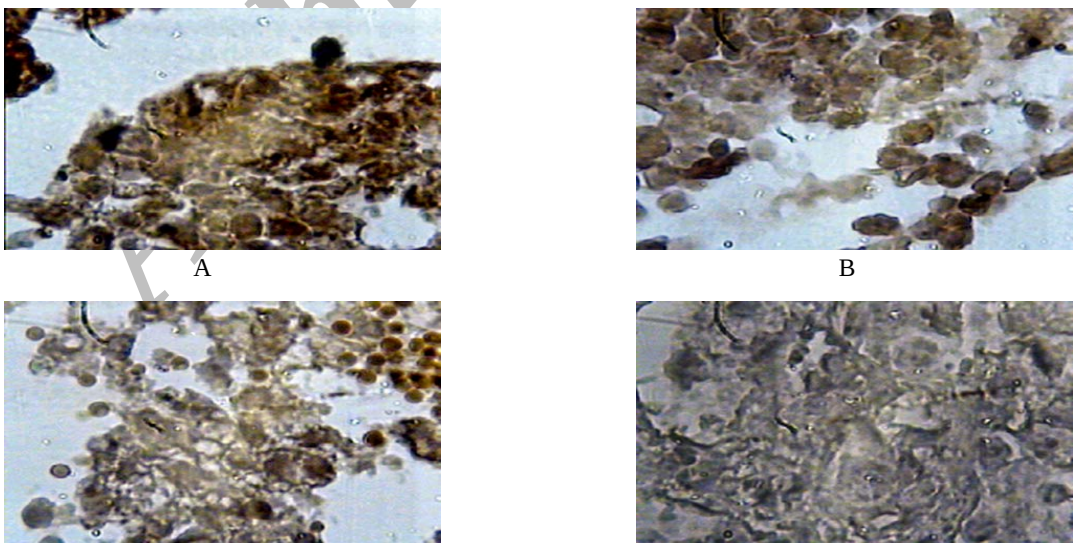
When the grade of tumors were proved by light microscope according to WHO format, cases and controls were selected based on their tumoral cell differentiation grade and anaplastic changes. Immuno-histochemistry studies for two markers, P53 mutant oncoprotein and Ki67 protein, was performed on blocks by using ABC method as described below. For analyzing the content of P53 oncoprotein and Ki-67 at nuclear membrane level, image cytometric method was used. This method is a dry computerized microspectrophotometric method using a CCD-camera adapted to Leitz microscopy and PIII hardware. The 410 nm filter was used for detecting the best light absorbance. The DAB deposition on nuclear membrane for P53 was divided in two separate group; those having the OD less than 300 pixels were graded as 1+ [group light (L)] and those having more than 300 pixels were graded as 2+ [group heavy (H)] (17). The same tabulation was used for Ki67 at nuclear cytosol stained by DAB. P53 mutant oncoprotein was detected by DO7 monoclonal antibody and kit PAb180l (DAKO) which detects only the mutant P53 protein, and Ki67 by DAKO. Avidin-Biotin method and DAB as chromogen were used in both tests. Agreement of two markers P53 and Ki67 was tested by Kappa. Odds ratio for each factor was calculated with its confidence interval at significance level of 5%.

RESULTS

Case group included 19 patients, 17 of them (%89.5) had P53 level of 2+; remaining 2 (%10.5) had P53 level of 1+. Rates of Ki67 were 2+ in 9 patients (%47.4) and 1+ in 7 patients (%36.8). Findings of 3 other patients (%15.8) were nonspecific and non-diagnostic (Fig. 1).

Control group included 31 patients, 11 of which (%35.5) had P53 level of 2+, 18 patients (%58.1) had P53 level of 1+; findings in 2 patients (%6.4) were nonspecific. Rates of Ki67 were 2+ in 3 patients (%9.7) and 1+ in 23 patients (%74.2); findings were nonspecific in 5 others (%16.1).

P53 and Ki67 markers had well concordance in all patients; %40.5 had level of 1+, and %25 had level of 2+ (Kappa = 0.257, $P < 0.007$), (Table 1). Results showed that low expression of mutant P53 in controls is accompanied by low proliferation index (low Ki67) and increased intensity of mutant P53 on nuclear membrane is accompanied by increased proliferating index (odds ratio = 13.9 , CI [%95]= 2.7-72.1) and Ki67 (odds ratio= 9.9, CI [%95]= 2.1-46.7) (Table 2). Lower expression of P53 and Ki67 had the same concordance.



A B C D

Fig.1. Samples of positive and negative staining. A and B, 2+ P53; C, 2+ Ki67; D, negative P53.

Table 1. Agreement of P53 and Ki67

	P53 1+	P53 2+	Total
Ki67 1+	17	11	28
Ki67 2+	2	10	12
Total	19	21	40

DISCUSSION

Various studies have shown the role of genetic alterations in neoplastic cells (1, 7). Among them, p53 as a suppressor gene is well studied in the last decade (14, 8). It can induce many form of human cancers through three processes (1, 2); homozygous loss, inactivation of both p53 alleles and inheritance of one mutant allele predisposing to inactivation of the second one. Furthermore, some investigators have found positive correlation between these genetic disorders predisposing to cancer and some environmental agents like tobacco, alcohol and coal (18-20). Concurrent appearance of Ki67 and P53 shows that by omission of suppressor effect of p53, the proliferation rate rises autonomically and that's just when we find the cell full of mutant P53 protein accompanied by Ki67 nuclear antigen detecting by their monoclonal antibodies (21). Only exceptions are complete omission of two p53 alleles and conditions with a high proliferation rate out of the p53 control, which are not concern of this study.

Hollstein *et al.* (1), Esposito *et al.* (7) and Kleihues *et al.* (14) have shown that p53 mutation is inherited in some families and carriers are at risk of malignant process, mean age of which differs from 16 years for sarcoma to 50 years for lung cancer. They believe this process to be entirly endogenous but many others including Rom *et al.* (3), Leopardi *et al.* (21), Shibe *et al.* (18) and Ahrendet *et al.* (20) suggest the role of environmental mutagenic factors such as DNA viruses. Boggs *et al.* (19) reported over-expression of P53 protein in sputum of lung cancer patients who had been exposed to coal smoke. Ahrendet *et al.* have shown that alcohol impairs the action of tumor suppressing gene of p53 in smokers with lung cancer (20) and researchers believe that there are a number of possible mechanisms responsible for alcohol's ability to increase the risk of p53 mutations in smokers developing lung cancer. Nichols *et al.* (4) and Birch *et al.* (22) believe that a dominantly inherited syndrome of p53 mutation promotes a wide spectrum of early onset cancers. Giatromanolaki *et al.* showed that angiogenesis is under control of p53 but there was no correlation between histology, grade, proliferation index (Ki67) and P53 expression in 107 operable NSCL cancers and vascular proliferation was independent to P53 expression (2).

Table 2. Risk of tumor cell differentiation for level of P53 and Ki67

	Tumor cell differentiation		Odds ratio	CI (95%)
	Low grade	High grade		
P53 1+	18	2	13.9	(2.7-72.1)
P53 2+	11	17		
Ki67 1+	23	7	9.9	(2.1-46.7)
Ki67 2+	3	9		

Considering proliferation marker (Ki67), Soomro *et al.* have shown that among 105 surgical cases of NSCLC, only patients with Ki67 scores of less than 5% did survive significantly longer than the rest (15). Histology had no predictive value in determining prognosis in both operable and inoperable groups; in a follow-up with mean duration of 20 months, Ki67 antibody was promising in identifying low and high grade disease in the initial stages of lung cancer. Nguyen *et al.* found that p53, bcl-2 and Ki67 expression is seen in 30%, 69% and 39% of NSCLC cases, respectively (23). They concluded that none of the markers can be used as an independent prognostic factor, whereas combination of them such as P53 positivity + low Ki-67 expression, P53 positivity + lack of cyclin-Dl expression and bcl-2 positivity + low Ki67 have a favorable prognostic value. Franciosi *et al.* showed that P53 and Ki67 were positive in 53% and 50% of FNA samples of lung tumors, respectively and this measurement can be used as a low invasive procedure before treatment planning of NSCLC (24).

In conclusion, our findings suggest that prevalence of P53 in NSCLC grows quantitively

according to the tumor grade, and just concurrent to the Ki67 marker. So detecting the mutant P53 protein in NSCLC not only can play an important role as a morpho- pathologic determinant of its grade (11), but also as a criterion of its proliferating manner. It can also be probably used for monitoring and predicting the stage of disease and at least helps planning therapy.

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