The Expression of P53 in Non Small Cell Lung Cancer

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ABSTRACT:

BACKGROUND:

Despite major advances in cancer treatment in the past two decades, the prognosis of patients with lung cancer has improved only minimally. Although tumor stage is the most significant prognostic factor, the variation in survival within staging groups requires information about additional factors influencing the outcome. Among several genetic aberrations that have been implicated in lung cancer, mutations in the p53 gene are the most common.

OBJECTIVE:

The aim of this study was to evaluate the expression of p53 in non-small cell lung cancer. To correlate the relation of p53 with some clinico-pathological parameters. And to compare the results with that of others.

PATIENTS AND METHODS:

Tumor tissues from 52 patients with non small cell lung cancer (NSCLC) were assessed by immune-histochemistry for the expression of p53. The immunohistochemical study was performed on formalin-fixed, paraffin-embedded sections using LSAB immune-peroxidase method.

RESULTS:

Thirty nine (75%) of 52 patients revealed aberrant immunostaining for p53. No significant relation was observed between the p53 and patient age (P=0.596). A significant association (P=0.048) was found between the p53 and the sex of the patient with higher expression in males and the p53 was significantly (P=0.0345) associated with histopathological type of tumor. Comparing p53 expression with grade resulted in a strong positive correlation (P= 0.0002). The percentage of p53-positive tumors progressively increased from (2.56%) in well differentiation to (53.85%) in poorly differentiated tumor.

CONCLUSION:

P53 was significantly association with sex, grade and histological type, the detection of p53 may be important marker to predict the prognosis of the patients with NSCLC and for stratifying these patients into more accurate prognostic group.

KEYWORDS: non small cell lung cancer, P53 expression, immune-histochemical study, prognosis.

INTRODUCTION:

Lung carcinoma is the most frequent cancer around the world and it is the leading cause of cancer death in Europe and the United States, and the same trend is seen in many other countries[1,2]. Lung cancer is clinically classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) [1,3]. NSCLC represents a heterogeneous group of cancers, consisting mainly of squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma and it represents approximately 80% of human lung cancers [1,3].

Despite recent advances in surgical and chemo/radiation therapies, the prognosis of lung cancer in general is poor[3], with Five-year survival is achieved by 10-15% of patients[1,3,4], this is due to rapid and extensive metastasis. The TNM staging system for lung cancer is widely used as a guide to predict prognosis, however patients at the similar stage of disease show marked differences in probabilities of survival. This illustrates the need to identify new prognostic factors that may help clinicians to better assess the survival probability and to optimize therapeutic efforts for each individual patient[2].

The development of NSCLC involves multiple genetic abnormalities that lead to malignant transformation of the bronchial epithelial cells, followed by invasion and lymph node and distant

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metastases. Among such genetic abnormalities, the p53 tumor suppressor gene appears to be the most frequent target, and abnormality of p53 plays an important role in the tumorigenesis of lung epithelial cells[1] .

P53 is a 53-kD nuclear phosphoprotein produced by the p53 tumor suppressor gene, which is localized on human chromosome 17p13. It has three main physiological functions: cell cycle regulation, induction of apoptosis and stabilization of the genome [2]. The mutations of the p53 gene occur in about 50% of NSCLC[3]. As p53 mutants are present in almost half of NSCLC whose incidence rate increasing every year, the possibility of abolishing their oncogenic effects is undoubtedly important for a successful treatment of NSCLC[3] . However, despite the large number of studies, the relation between the p53 alterations and survival in NSCLC has been controversial[2].

PATIENTS AND METHODS:

This is a retrospective, case-series study. Fifty-two cases which were diagnosed as primary lung cancer (non small cell type) in Al-Jamhori Teaching Hospital in Nineveh province in Iraq during a period extending from November 2010 to May 2012 were collected and included in this study. Sections from paraffin embedded tissues were taken and stained with Hematoxylin and Eosin, then examined under the light microscope. Histological type and degree of differentiation were defined according to World Health Organization criteria. Other informations which included in this study (age and sex of the patients) were retrieved from the medical reports.

Immunohistochemical staining: A 4-µm section was obtained from formalin-fixed, paraffin embedded tissue of each of these 52 samples of NSCLC. The procedure for the IHC detection of p53 was performed following the instruction provided by the manufacturer. The material for the procedure was obtained from Dakocytomation by using the streptavidin-biotin-peroxidase complex method . The sections were briefly immersed in citrate buffer (0.01 mol liter citric acid: pH 6.0) and incubated for 5-minute intervals at 100^{o}C in a microwave oven for antigen retrieval (Target Retrieval Solution, High pH, code No. S 3308). They were then incubated with p53 antibody (Monoclonal mouse antibody provided in liquid form as cell culture supernatant Clone: DO-7, code No. M 7001. Isotype: IgG2b, kappa) diluted at 1:100, in phosphate-buffered saline (PBS). To detect bound antibodies, the Universal detection DAKO LSAB™+/HRP kit (code No. K 0679) containing secondary antibody and streptavidin-biotin was used. Negative controls were made by omitting the primary antibody during the process of immunohistochemical staining. The positive controls were a known p53-positive breast cancer.

Immunohistochemical evaluation: The immunostaining of p53 protein appear as brown nuclear stain. All slides were evaluated for this immunostaining by light microscopy using a Leitz dialux microscope. The percentage of positive cells was calculated by counting more than 1000 cells in randomly-chosen high-power fields (10 x40) and was scored using a visual grading system based on the number/ percentage of positive tumor cells graded on a scale of 0 to 3+:

0% of positive cells = 0; 1-10% = 1+; 11-90% = 2+; 91-100% = 3+. The sections were considered to be negative when the proportion of the stained cells was 0 and 1+(less or equal to 10%). whereas only 2+ and 3+ (over 10%) samples regarded as positive. The criteria was based on the findings of other reports [1 ,2,5,6,7], and studies found that the concordance rate between the DNA analysis of p53 and IHC staining of it (p53 protein) was relatively constant when this cut-off level used [5,6].

Statistical analysis: The associations between expression of p53 and characteristics of patients were analyzed by the using of Fisher exact test. The P value of <0.05 was considered statistically significant[2].

RESULTS:

Fifty two cases of primary lung cancer (non small cell type) were included in this immunohistochmical study of p53 protein. The mean ages of those cases was 61.6 years ranging from 41 to 77 years; they were segregated into 2 groups (<60 and ≥60 year) with15 cases were less than 60 years of age and 37 were 60 years or older.

For the sex, there were 7 female (13.5%) and 45 male (86.5%) patients (female :male =1: 7.4). The tumors were included 31(59.615%) squamous cell carcinomas, 11(21,15%) adenocarcinomas, 8(15.385%) large cell carcinoma, and 2cases (3.85%) with adenosquamous type. See figures (3,4,5,6,7,8).

Most of the tumors were either moderately differentiated 18 cases (34.62%) or poorly differentiated 27cases (51.92%) with only 7 cases (13.46%) being well differentiated. Table(1) summarises the main characteristics of the patients.

The p53 protein positive expression was detected in 75% (39/52) of the patients. No significant relation was observed between the p53 and patient age (P=0.596), the higher positivity of p53 (74.36%) was found in patient age ≥60 year, as shown in figure (1). A statistically significant association (P=0.048) was found between the p53 and the sex of the patient with higher expression in males. It was positive in 35(89.74%) and in 4(10.26%) of the male and female respectively, as shown in figure (2).

Regarding the histological types of tumor, a statistically significant association was found (P=0.0345). In this study higher percentage of p53 positivity (58.974%) was found in the squamous cell carcinoma than other histological types, as shown in table (2). Comparing p53 expression with tumor grade resulted in a positive direct correlation (P= 0.0002). In well differentiated tumor (n = 7), 1 case (2.56%) was p53 positive, in moderately differentiated (n = 18) 17 cases (43,59%) were positive and p53 was present in 21(53.85%) of poorly differentiated tumor (n = 27). The percentage of p53 positive tumors progressively increased from (2.56%) in well to (53.85%) in poorly differentiated tumors. as shown in table (3) & figures (3,4,5,6,8).

Table 1: Clinicopathological parameter of 52 cases of NSCLC.

	No. of cases	(%)
Age in years		
<60 year	15	28.85%
≥60 year	37	71.15%
Sex		
Female	7	13.46%
Male	45	86.54%
Histological types		
Squamous cell carcinoma	31	59.615%
Adenocarcinoma	11	21,15%
Large cell carcinoma	8	15.385%
Adenosquamous	2	3.85%
Grades		
Well	7	13.46%
Moderate	18	34.62%
poor	27	51.92%

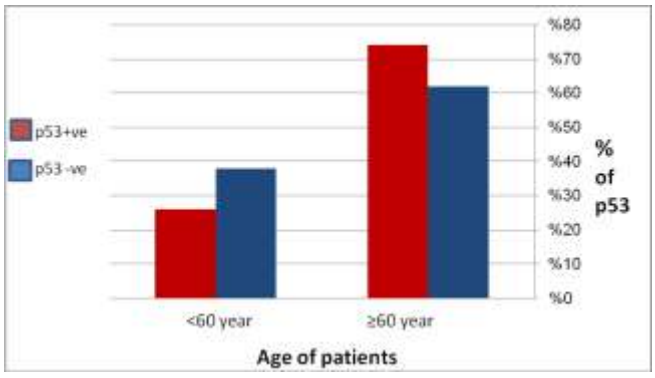


Figure 1: The relation of p53 expression with the age of the patients.

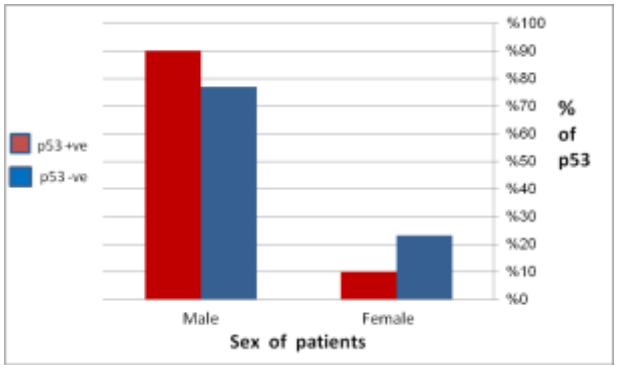


Figure 2: The relation of p53 expression with the sex of the patients.

Table 2: The relation of p53 expression to the histological types of NSCLC.

Histological types	No. of cases (%)	P53 expression positive(%) negative (%)	P- value
Squamous cell carcinoma Adenocarcinoma Large cell carcinoma Adenosquamous	31(59.615%) 11 (21,15%) 8(15.385%) 2(3.85%)	23(58.974%) 8(61.54%) 8(20.513%) 3(23.08%) 7(17.949%) 1(7.69%) 1(2.564%) 1 (7.69%)	0.0345

Table 3: The relation of p53 expression to the grades of NSCLC.

Grades	No. of cases (%)	P53 expression positive(%) negative (%)	P- value
Well Moderate poor	7(13.46%) 18(34.62%) 27(51.92%)	1(2.56%) 6(46.154%) 17(43,59%) 1(7.692%) 21(53.85%) 6(46.154%)	0.0002

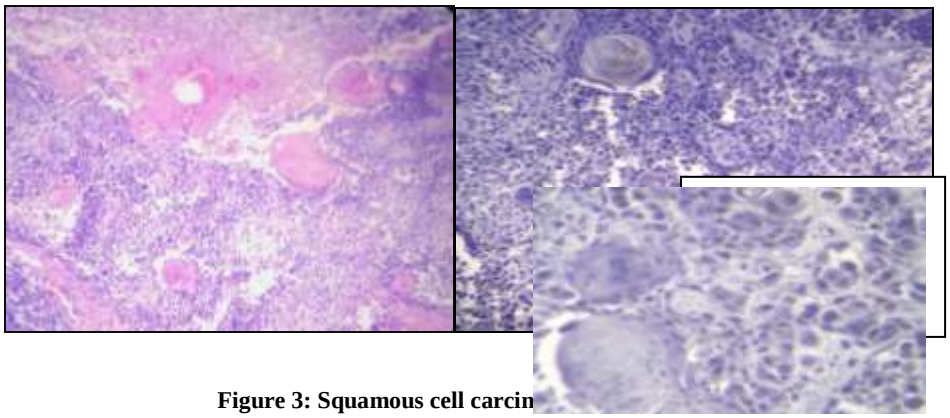


Figure 3: Squamous cell carcin
Left,(X 100) H&E; right, (X 100) p53 negative.

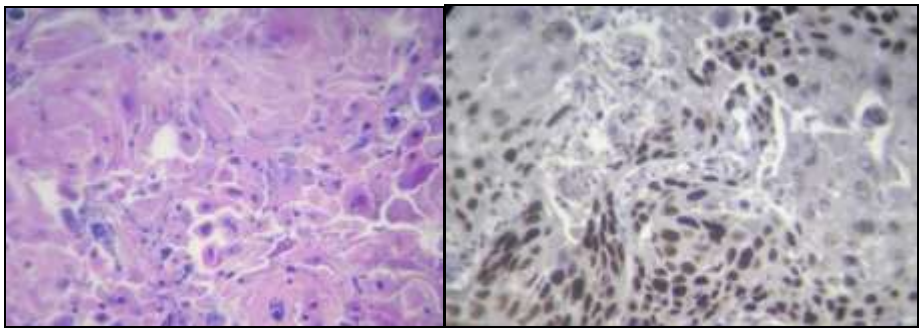


Figure 4: Squamous cell carcinoma (moderate differentiation)
Left,(X 400) H&E; right, (X 400) p53 positive .

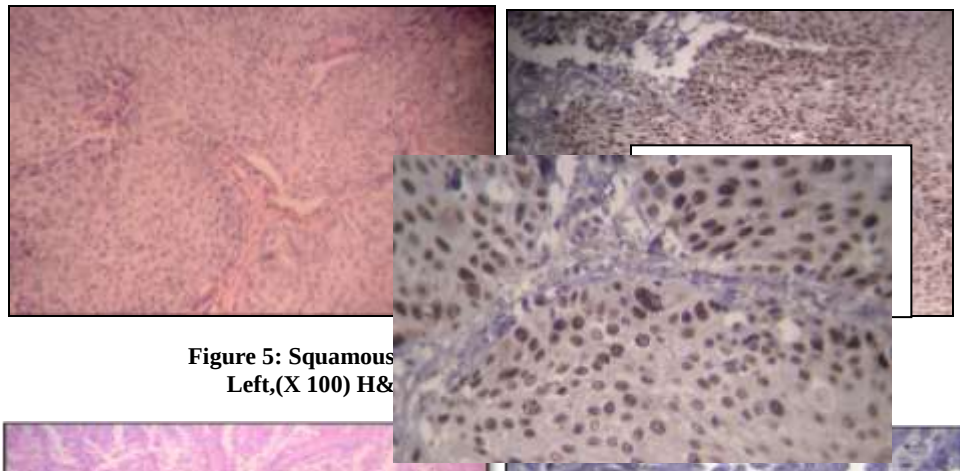
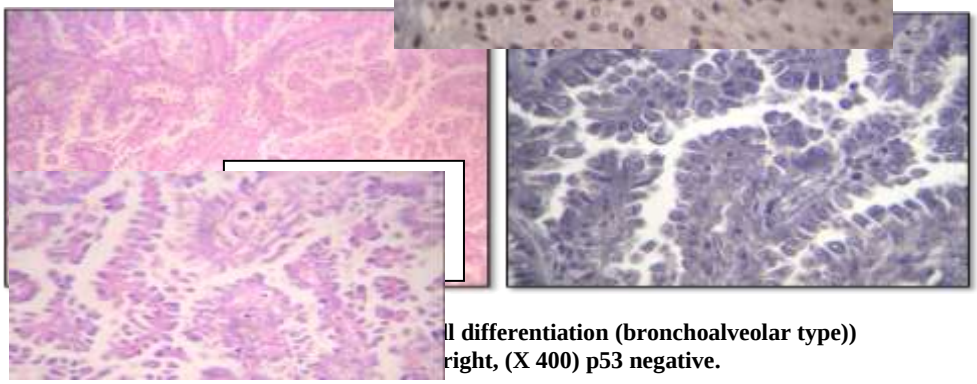


Figure 5: Squamous
Left,(X 100) H&



l differentiation (bronchoalveolar type))
right, (X 400) p53 negative.

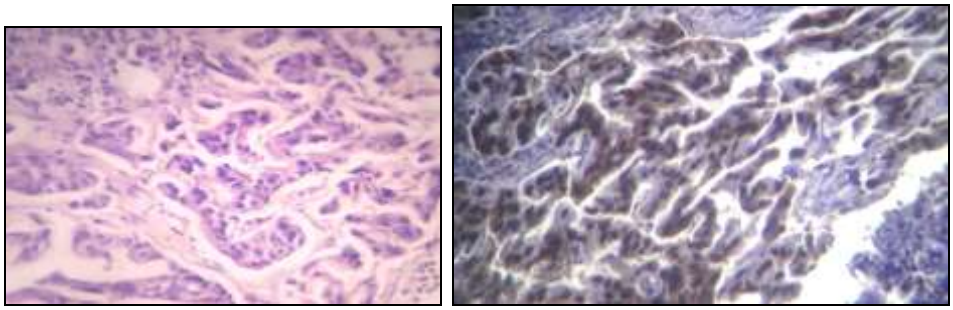


Figure 7: Adenocarcinoma (poor differentiation)
Left,(X 400) H&E; right, (X 400) p53 positive.

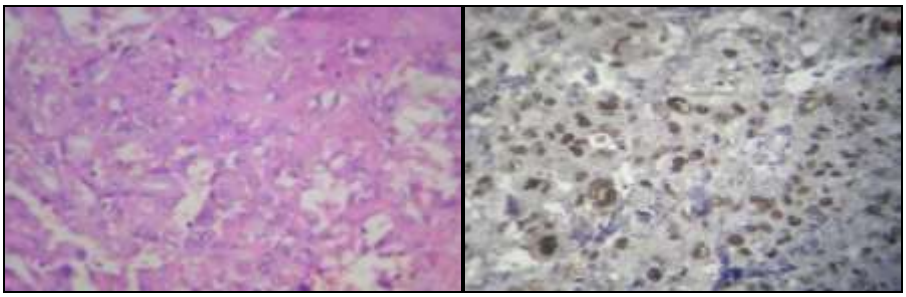


Figure 8: undifferentiated large cell carcinoma
Left,(X 400) H&E; right, (X 400) p53 positive.

Table 4: Comparison of p53% expression with that of other studies.

Study	Region	Year	No. of cases	P53 +ve	%
Current study	Iraq(Nineveh)	2012	52	39	75%
Rybárová S. et al[1]	Slovakia	2011	54	25	46.3%
Abdul-majeed B.A. et al[8]	Iraq(Baghdad)	2010	30	21	70%
Poposka B.I. et al[2]	Macedonia	2009	80	43	53,75%
Tsao M.S.[10]	USA	2007	253	132	52%
Uramoto H et al[4]	Japan	2006	132	52	39.4%
Esposito V.et al[11]	Italy	2004	68	44	64.7%
Yoo J.et al[9]	Korea	2004	147	107	73%
Poleri C. et al[12]	Argentina	2003	53	23	43.4%
Sheng Lai R. et al[13]	Japan	2002	114	59	51.8%

DISCUSSION:

Non-small cell lung cancers (NSCLC) comprise 80% of lung cancers[3]. p53 normally has a too short half-life to be detected by immunohistochemistry. However, mutant p53 protein has a prolonged half-life, which results in accumulation in the tumor cell nucleus, which in turn can be detected by immunohistochemical methods. Nuclear p53 protein immunoreactivity demonstrates a high level of corresponding mutation of the p53 gene, especially the missense type which is a quite frequent mutation[1]. This missense mutations alters the protein structure, inhibiting rapid degradation of the protein. Therefore, accumulation of p53 protein was suggested to indicate the presence of p53 gene mutations. The prognostic value of p53 expression is still unclear and controversial[1].

Among the 52 cases with NSCLC which were included in the study, p53 was positive in 39 (75%) cases. The result of this study is slightly similar to that found by Abdul-majeed B.A et al[8] and Yoo J.et al[9] which were (70%) and (73%) respectively, but it was higher than the rate of detection found in other studies, ranging from 39.4% to 64.7%, as shown in table (4). This variation or difference may be due to difference in grade of the tumors that observed in this study, that only 7 cases (13.46%) were diagnosed as well differentiated while 45 cases (86.54%) were diagnosed as moderate and poorly differentiated tumor. It may also be due to difference in the histological types and variation in patient population included in the studies.

No significant association was found between the p53 expression and the age of the patients (P=0.596). This result in agreement to that found by Poposka B I et al[2], Uramoto H et al[4], and Berghmans T et al[7]. While Ahrendt A.S et al[14] found a direct relation of p53 with the age of the patients.

A significant association (P=0.048) was found between the p53 and the sex of the patients with higher expression in males, this may be due to that in this study 86.5% (45/52) of the cases were males and the p53 was positive in 75% (39/52) of the all patients. This result is

incomparable to that found by Berghmans T et al[7], Tsao M.S[10] and Ahrendt A.S et al[14]. However Poposka B I et al[2], Uramoto H et al[4] and the Yoo J.et al[9] found no relation between the p53 expression and the sex of the patient.

The p53 expression was significantly associated with the histological type of the tumor (P=0.0345), this expression was found to be higher (58.974%) in squamous cell carcinoma than in other histological types. This result is similar to that found by Abdul Mjeed B.A. et al[8] who was also found that a high positivity of p53 (72.2%) in squamous cell carcinoma. Also These results are compatible with other previously published reports [1,2, 4,10,14] . So, It may be possible that, stabilized p53 protein plays an important role in squamous cell lung cancer development [1]. Berghmans T. et al[7] and Yoo J. et al[9] found there is no relation of p53 expression and histological type. This may be attributed to the heterogeneity of retrospective studies, differences in immune-histochemical methods used as well as the laboratory design.

A significant directed relation (P=0.0002) between expression of p53 protein and grading of tumor was reported. That there is increasing of the expression of p53 with the increasing of the tumor grade. Similar association was noticed by Rybárová S. et al[1], Abdul-majeed B.A et al [8] and Ahrendt A.S et al[14] this result is due to that p53 gene is known to have a proto-oncogenic state of most cancers as well as NSCLC. The Mutation of p53 will lead to the removal of its oncosuppresor effect, so that the cell will be allowed to accept more and more mutations, above DNA repair system capacity, and this progresses to poor differentiation[15].

Saber S. and Salehian P. [15] they did a study of p53 and Ki-67 in NSCLC and they found that p53 in NSCLC grows quantitively according to the tumor grade, and just concurrent to the Ki67 marker. And the detecting of the mutant p53 protein in NSCLC not only can play an important role as a morpho- pathologic determinant of its grade, but also as a criterion of its proliferating manner. That is to say with the presence of p53 mutation, cell proliferation system will act autonomically out of control. So theoretically speaking, a poorer differentiation grade is associated with a higher proliferation rate [15].

Tsao M.S. et al[10] did a large prospective study of the p53 expression on 253 cases of NSCLC in Chicago. They found that the patients with p53-positive tumors had significantly shorter survival than did those with p53-negative tumors, indicating that p53 protein over expression is a significant marker of poor prognosis [10]. Even after they did multivariate adjustment for other potential prognostic factors the result remained statistically significant .

Similarly, in some other studies survival has been adversely affected by p53 gene mutation and/or protein overexpression [2,14, 16,17,18,19].

However some authors found that there was no relationship between p53 alterations and survival [5,7,11,12]. The controversial results are difficult to interpret: they may significantly vary depending on the different method used, such as the use of nucleic acid-based screening versus immunohistochemistry, and in immunohistochemistry, the use of different antibodies, different procedures and different cut of values.

CONCLUSION:

In this study the p53 had a statistically significant association with sex, grade and histological type. These findings are potentially important for prognostic reasons, so that using immunohistochemical staining of p53 as clinical biological marker may predict patients with NSCLC with poor prognosis and help for stratifying patients with NSCLC into more accurate prognostic groups.

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