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MEASURMENT AND EVALUATION OF SERUM ANTI P53 ANTIBODY LEVELS IN PATIENTS WITH LUNG CANCER AT ITS INITIAL PRESENTATION

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Abstract

The present study aimed to detect and evaluate anti p53 Ab in patients newly diagnosed lung cancer and its relation to histological type, stage of the disease and response to therapy. For this purpose , 48 lung cancer patients at first presentation, 15 patients proven to have pulmonary disease other than lung cancer and 10 apparently healthy subjects were selected for this study. Ten patients from lung cancer group were followed up after treatment. ELISA procedure was used to detect serum anti p53 antibodies in all subjects .Results:There was statistically insignificant difference between cases with positive and negative anti p53 as regards the mean age and sex. Also percentage of positive anti p53 Ab cases in smokers and those accompanied with pleural effusion were significantly higher than in non smokers and those with no pleural effusion . It was found that 27% of lung cancer patients in this study were anti p53 Ab positive with no cell type difference, as there was no statistical significant difference between different histopathological types as regards the positivity of anti p53 (25% squamous cell carcinoma , 20 % adenocarcinoma ,33.3% large cell carcinoma and 25% small cell carcinoma) nor regarding the disease stage. Anti p53 Ab was never detected in association with pulmonary diseases other than carcinoma or in control sera . Ten patients (3 positive for anti p53 and 7 negative) were randomly selected where anti p53 was repeated after 6 months of chemotherapy that led to either partial or complete remission of disease ,one of them became negative, the other two positive cases showed reduced titer , in already 7 neg-

ative cases before treatment , none of them could find anti p53 Ab after treatment so anti p53 Ab could be a useful tool for oncologists in their attempt to analyze the patient response to therapy.

Introduction and Aim Of The Work

The p53 tumour suppressor gene is involved in control of the cell cycle, DNA synthesis and repair, cell differentiation, genomic plasticity and programmed cell death (Harris et al., 1993) .

Mutations of the p53 gene are currently the most common genetic alteration identified in human cancer (Levine et al., 1991 and Chang et al., 1995).

Mutant p53 proteins are known to be targets of host immune system (Crawfort et al., 1982). Examination of serum has shown that some patients with cancer that harbour a mutated p 53 allele have mounted a humoral immune response to abnormal level of p53 protein. Anti-p53 antibodies (Anti-p53) have been demonstrated in up to 26% of patients with various malignant conditions including breast, colorectal., and liver tumours as well as lymphoma (Crawfort et al. 1982., Green et al., 1994 and Chang et al. 1995).

The objective of this study is to detect and evaluate anti-P53 antibodies in patients newly diagnosed with lung cancer and its relationship to histological type, stage of the disease and response to treatment.

Subjects and Methods

Subjects:This study included 48 patients with lung cancer at first presentation. They were 37 males and 11 females, their age ranged from 33 to 73 years with a mean of 56.3 ± 9.75 years. They were selected from Benha University hospital and cancer institute of Tanta. Also 15 patients (9 males and 6 females), their age ranged from 38 to 69 years with a mean age of 55.5 ± 8.45 years proven to have pulmonary disease other than lung cancer. In addition to 10 apparently healthy subjects (7 males & 3 females), their age ranged from 35 to 73 years with a mean age of 49 ± 10.77 years. 10

patients (6 males & 4 females) selected from the first group for follow up, their age ranged from 48 to 73 years with a mean age of 62.3 ± 12.4 years. All cases were subjected to : Full history taking of the present illness, complete clinical examination : general examination & chest examination, chest X-ray, C-T chest examination, bronchoscopy and fine needle aspiration biopsy for pathological examination for diagnosis of lung cancer .Sera were obtained after diagnosis of cancer but prior to any treatment, for patients with follow up samples were obtained again 6 months after starting chemotherapy.Seven milliliters of whole blood were centrifuged at 3000 rpm for 15 min, and the supernatent was stored at -70°C until used for routine laboratory tests(liver & kidney functions) in addition to measuring the serum level of anti p53 antibodies in all patients and normal control .

Methods:

ALT and AST : by a kinetic method , IFCC, 1980

Urea : modified urease - Berthelot method (Patton et al, 1977).

Creatinine : by a kinetic method of Henry et al., 1972.

Anti p53 antibody ELISA : Dianova 2-generation (Segawa et al., 1998).

Serum samples were diluted 1:100 in the sample dilution buffer prepared in the kit before assay (calibrator and negative control used undiluted). The samples were added to 96 microtitre wells precoated with human recombinant p53 protein and incubated at 37°C for 1 hour. After washing, anti p53 antibodies that have attached to the protein of each well, were bound by peroxidase - conjugated goat antibodies IgG. Color was developed with the chromogenic substrate tetramethylbenzidine, and the absorbance of each well was determined using a microplate reader at a wave length of 450 nm (Bio-rad. USA). The cut off was calculated by multiplication of the absorption value of the calibrator (which was in this test equal to 2.1) with

a lot specific factor (the specific factor of this lot is 0.15), so cut off was 2.1x0.15= 0.315.

Quantification of P53 autoantibody titer of positive cases:

The antibody titer was measured with the help of a calibration curve. The antibody titer was measured in units [U]. 1 unit is defined as p53 binding activity which corresponds to the binding activity of the calibrator.

Results

This table showed that there was insignificant difference between cases with positive and negative anti P53 as regards the mean age (P value > 0.05).

This table showed that there was insignificant difference between cases with positive and negative anti P53 as regards sex distribution (P value > 0.05).

This table showed that smokers were statistically significant higher than non- smokers regarding the positivity of the anti P 53 (P value 0.006).

This table showed that cases with pleural effusion were significantly higher than those with negative pleural effusion as regarding the positivety of anti P53 (P value 0.009).

This table showed that there was insignificant difference between different histopathological types regarding the positivity of the anti P 53 (P Value > 0.05).

Histopathological classification was based on the World health organization criteria (WHO, 1982)

This table showed that there was insignificant difference between different stages regarding the positivity of the anti P53 (P value > 0.05).

The patients were staged using the tumor-node-metastasis system (Mountain et al 1986)

Table (1): Comparison between mean age of positive and negative anti p53 lung cancer patients .

	Anti P53	N	Mean	Std. Deviation	t	P value
Age(years)	**Positive**	13	57.7	8.6	0.8	> 0.05
	Negative	35	54.9	9.52		

Table (2): Sex distribution among patients with lung cancer as regards Anti P53.

Sex		Anti P53		Total	Chi-square	P value
		Negative	Positive			
Male	**Count**	26	11	37	2.1	>0.05
	% within sex	70.3%	29.7%	100.0%		
Female	**Count**	9	2	11		
	% within sex	81.8%	18.2%	100.0%		

Table (3): Comparison between smokers and non smokers in the studied subjects as regards Anti p53.

Smoking / Anti P 53	Nonsmokers		Smokers	Total	Chi-square	P value
Positive	No	2	11	13	6.2	0.006
	%	15.3%	84.7 %	100.0 %		
Negative	No	32	28	60		
	%	53.4%	46.6%	100.0%		