Evaluation of Serum LDH, p53 and BCL2 in Lung Cancer Patients

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Summary

To evaluate the role of serum lactate dehydrgenase (LDH) and the expressions of p53, BCL2 in non small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) patients and correlate their expressions with clinicopathological parameters and diagnostic lung cancer markers panel, prognostic role and disease outcome. Total 100 lung cancer patients having NSCLC and SCLC were enrolled in the study. p53 and BCL2 expression were studied by Immunohistochemistry method and serum LDH level were analyzed in Cobas 6000 analyzer.p53 expression was significantly higher in males, smokers, LN3 nodal status and in squamous cell carcinoma patients (SCC). A significant inverse correlation was noted with adenocarcinoma markers. Higher BCL2 expression was seen in patients with smoking habits, T4 and T2 tumor size and stage III disease. A positive correlation was noted in adenocarcinoma markers and an inverse correlation with SCC markers. In SCLC, a significant positive correlation was noted with chromogranin. A higher abnormal LDH level was noted in T4 and T3 tumor size, without lymph node involvement, stage III disease and in SCC patients. A higher incidence of death was observed in patients with abnormal LDH level. In SCLC, a significant higher abnormal LDH level was noted in patients with ≤56 years of age. No significant correlation was found with survival. In inter-marker correlation between p53, BCL2 and LDH showed that patients with p53 and BCL2 positive had high LDH level. In conclusion, higher expression of mutant p53 and BCL2 in smokers suggests that higher tobacco consumption increased the risk of mortality or poor survival by inducing the altered metabolism of p53 and BCL2 by effect of carcinogens. A positive correlation of mutant p53 and BCL2 expression with LDH in adenocarcinoma as well as impact of LDH on survival suggests that LDH plays an important role in cancer cell metabolism.

Keywords: p53, BCL2, LDH, NSCLC, IHC, SCLC

Introduction

Lung cancer is the leading cause of cancer incidence and mortality worldwide. In India, the incidence of lung cancer is 5.9% and mortality is 8.82%.[1] At Gujarat Cancer & Research Institute a regional cancer center of Western India, incidence of lung cancer is accounted for 5.8% according to hospital-based cancer registry.

Lung cancer is broadly divided into small cell lung carcinoma (SCLC) and non small cell lung carcinoma (NSCLC), with a rapid frequency of proliferation in both smokers and non-smokers.[2] The variation in the rates of lung cancer unfold the maturity of the tobacco epidemic and differentials in the historic patterns of tobacco exposure, including intensity, the time period of smoking, type of cigarettes, degree of inhalation and environmental pollution.[3-4] Besides, tobacco consumption, other factors such as genetic susceptibility, poor diet, occupational exposures and air pollution may act autonomously in shaping the illustrative epidemiology of lung cancer.[5]

Apart from the above parameters, one of the major causes which induce various types of cancers including lung cancer is altered metabolism which is an emerging and potential hallmark of cancer and plays an important role in the cancer cell progression. It promotes the tumor formation by triggering various oncogenes such as Ras, RAF, EGFR, MIC, MYB, ABL2, BCR and tumor suppressor genes such as p53, FLT3, BRCA1, BRCA2, IDH1 and many more. Different intracellular metabolic enzymes like LDH (Lactate dehydrogenase) are released by tumor cells, due to intracellular machinery alteration and apoptosis deregulation.[6] The signaling pathways perturbed in cancer regulates metabolism with some metabolic enzymes functioning as tumor suppressors genes and oncogenes.[7]

In lung cancers, altered metabolic genes are ubiquitously either over expressed or under expressed. The mechanism of altered metabolism was first explained by Otto Warburg who observed an abnormal characteristic of cancer cell energy metabolism in which only 2 ATP molecules are produced by enzyme named Lactate dehydrogenase (LDH) which interconverts pyruvate to lactate at the end of the glycolytic pathway using NAD+ as a cofactor. Down regulation of LDH can lead to an inhibition of cancer cell proliferation.

Uncontrolled proliferation of cancer cells induce the expression of p53 which is known as the guardian of genome regulates many different aspects of metabolism. The function of p53 in regulation of metabolism includes the regulation of glycolysis, pentose phosphate pathway, mitochondrial oxidative phosphorylation and lipid metabolism. p53 gene is frequently mutated in maximum numbers of human tumors. p53 lost its tumor suppressive function and tumor associated mutant p53 proteins often gain new tumorigenic activities termed as gain-of-function (GOF) of mutant p53.[8] It has been reported that mutant

p53 proteins and wild-type p53 proteins frequently regulate similar cellular biological processes with contradictory effects.[8-9] For example, in metabolic regulation, wild type p53 inhibits the initiation of glycolysis whereas; mutant p53 promotes glycolysis through different mechanisms.

BCL2 is the anti-apoptotic protein that localizes to the mitochondria and blocks the recruitment and activation of pro-apoptotic proteins such as Bax. p53 is proposed to activate cell cycle check points, whereas anti-apoptotic gene BCL-2 has shown to inhibit cell death. The widen roles of BCL-2 proteins in energy metabolism come up with its additional ways in which this molecule alters normal metabolism beyond its well-recognized role in regulation of apoptosis.[10]

So, the present study aimed to evaluate the role of p53, BCL2 and serum LDH in Lung cancer patients to predict the role of altered metabolism and correlate its activity p53 and BCl2 and to evaluate its individual and combined efficacy in predicting prognosis of NSCLC and SCLC patients.

Material and Method

In this retrospective study, 100 (80 patients with NSCLC and 20 patients with SCLC) lung cancer patients who had been diagnosed and treated at Gujarat Cancer & Research Institute (GCRI)in the duration of 2015 to 2017 were included. The detailed clinical history such as patient's age, gender, habit (smoking or tobacco), histopathological finding, and treatment offered and disease status were recorded in the division from the case file maintained at the Institutional Medical Record Department. Paraffin embedded tissue block of these lung cancer patients were archived from Histopathology Department of GCRI. The study was approved by the Institutional Scientific Review Board and Ethics Committee.

Immunohistochemical Localization

Immunohistochemical localization of p53 and BCL2 were evaluated on formalin fixed paraffin embedded (FFPE) tissue blocks containing primary tumor evaluated by Hematoxylin and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). The commercially available antibodies used were p53 (Clone SP5, Thermo Scientific. 1:50) and BCL2 (Clone 124, Cell Marque. 1:100). Briefly, 3-4 µm thin sections were cut on microtome (Leica, Germany) and taken on to 3-Aminopropyltriethoxysilane (APES) coated slides. Briefly the protocol included following steps of deparafinization using EZ prep solution, antigen retrieval for 30 minutes for BCL2 and 90 minutes for p53 using retrieval solution CC1 and incubation with ultra view DAB inhibitor for 4 minutes, addition of 100µL of p53 and BCL2 antibody at 37ºC for 120 minutes 32 minutes respectively, followed by incubation with ultra view HRP multimer for 8 minutes, ultra view DAB Detection kit for 8 minutes. The sections were counterstained with hematoxylin for 8 minutes and bluing reagent for 4 minutes and mounted with DPX.

Scoring

Two individual observers scored the sections. Cytoplasmic staining pattern for BCL-2 and nuclear staining pattern was observed for p53. Histoscore (H-score) was evaluated by multiplying percentage of positive cells with the staining intensity. H-score from 0 to 300 was evaluated where score of less than or equal to 50 (≤50) was scored as negative and that of more than 50 (>50) was scored as positive for p53 as well as BCL2.

Evaluation of Serum LDH

Evaluation of lactate dehydrogenase in human serum was done on Roche/Hitachi Cobas C systems: Serum collected using standard sampling tubes. Reagents are ready to use and packed in closed cassettes it makes reagent handling fully automated. This method has been standardized against the original IFCC formulation using deionized water as zero calibration pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity. The COBAS 6000 system automatically calculated the LDH activity of each sample.

Statistical Analysis

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Mean, standard error (SE) of mean and median were calculated. Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. Survival analysis was performed using Kaplan-Meier survival function and the differences in survival were tested for statistical significance using log-rank statistic. P values ≤ 0.05 were considered to be significant.

Results

Expression of p53, BCL2 and Serum LDH in lung cancer patients

In NSCLC, 39% (31/80) showed nuclear expression of p53 where as 61% (49/80) were negative for p53 expression (Figure: 1a and 1b). Cytoplasmic expression of BCl2 was observed in 35% (28/80) of patients and 65% (52/80) of patients were negative for BCL-2 expression (Figure: 2a and 2b). Out of 80 patients 56% (45/80) patients showed abnormal LDH level, while 44% (35/80) patients showed normal (normal range 100-190 IU/L) LDH level.

In SCLC, all the patients showed positive nuclear expression of p53 hence 100% positivity was noted, Cytoplasmic expression of BCL2 found positive

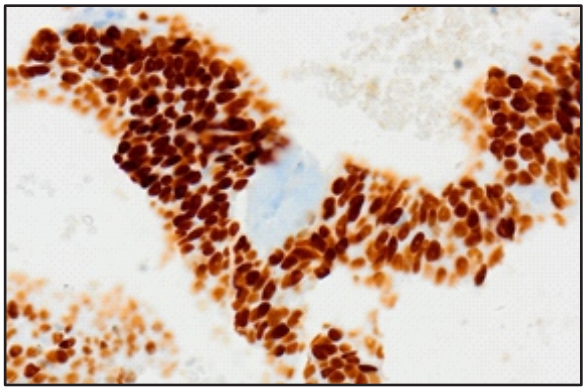

Figure 1(a): p53 positive expression in adenocarcinoma patients (Nuclear staining)

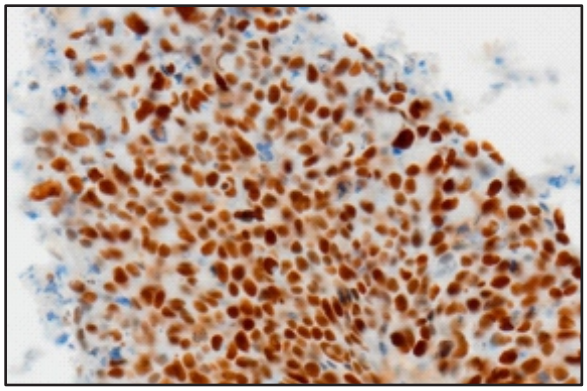

Figure 1(b): p53 positive expression in squamous cell carcinoma patients (Nuclear staining)

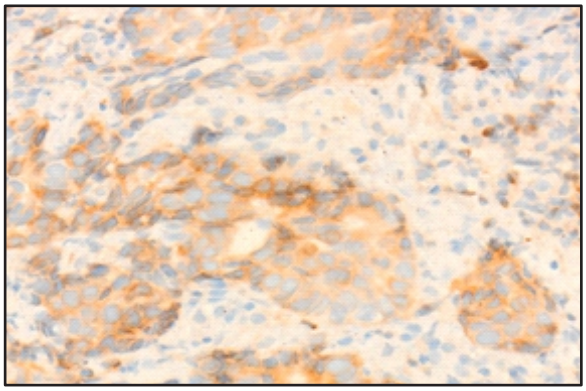

Figure 2(a): BCL-2 positive expression in adenocarcinoma (cytoplasmic staining)

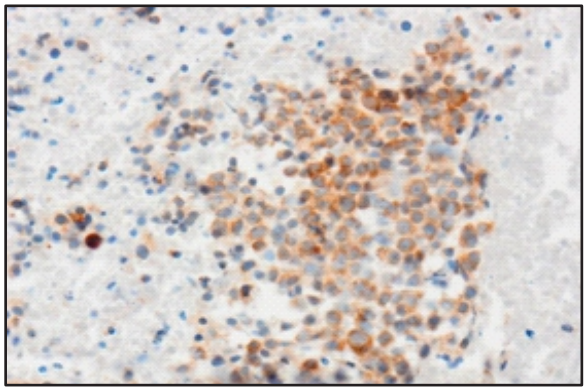

Figure 2(b): BCL 2 positive expression in squamous cell carcinoma (cytoplasmic staining)

in 45% (9/20) of patients and 55% (11/20) of patients showed negative BCL2 expression. Out of 20 patients 70% (14/20) patients showed abnormal LDH level, while 30% (6/20) patients showed normal LDH level.

Correlation of p53, BCL2 and Serum LDH with Clinical and Pathological parameters

In relation with clinical parameters, a trend of higher p53 expression was noted in patients with >60 years of age (49%, 18/31). A significant higher p53 expression was observed in male patients (43%, 31/31; p=0.018) and in smokers (55%, 26/31) compared to non-smokers (15% 5/31; p=0.0001). Correlating p53 expression with pathological parameters, a significant higher expression of p53 was noted in patients with LN3 (nodes represent contralateral mediastinal or contralateral hilar lymphadenopathy or scalene or supraclavicular nodes)nodal status (85% 11/31) as compared to patients without lymph involvement (43%, 3/31), LN2 (nodes represent ipsilateral mediastinal or subcarinal lymphadenopathy) (29%, 16/31) and LN1 (nodes are ipsilateral nodes within the lung up to hilar nodes) (20%, 1/31; p=0.002) involvement. Further, higher p53 expression was noted in patients with stage III (39%, 29/31) disease as compared to patients with stage II (20%, 1/31) disease. There was only one patient with stage I (1/31) disease who showed p53 expression. With histological sub types a significant higher p53 expression was observed in patients with squamous cell carcinoma (60%, 24/31) as compared to patients with adenocarcinoma (17% 7/31; p=0.0001). (Table 1a)

No significant correlation of BCL2 was noted with any clinical parameters however, higher BCL2 expression was observed in smokers (40%, 19/28). A trend of higher BCL-2 expression was noted in patients with stage III (35%, 26/28) disease as compared to patients with stage II (20%,1/28) disease, There was only one (1/28) patient was in stage I disease and showed BCL-2 expression. (Table 1a)

No significant correlation of LDH was noted with clinical or pathological parameters. However, higher LDH level was noted in patients with stage III (57% 42/45) disease as compared to patients with stage II (40%, 2/45) disease. There was only one patient with stage I disease whose LDH level was found to be abnormal. A higher trend of abnormal LDH level was noted in patients without Lymph Node involvement status (71% 5/45) as compared to patients with LN2 (nodes represent ipsilateral mediastinal or subcarinal lymphadenopathy) (58%, 32/45), LN3 (nodes represent contralateral mediastinal or contralateral hilar lymphadenopathy or scalene or supraclavicular nodes) (46%, 6/45), and LN1 (nodes are ipsilateral nodes

Table 1 (a): Correlation of p53, BCL-2 and Serum LDH with clinical and pathological parameters in NSCLC patients (N=80)

Parameters	N (%)	p53 expression		BCL-2 expression		LDH level	
	N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
Age(years)		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
≤60	43(54)	30(70%)	13(30%)	26(61%)	17(39%)	21(49%)	22(51%)
>60	37(46)	19(51%)	18(49%)	26(70%)	11(30%)	14(38%)	23(62%)
Gender		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
Male	72(90)	41(57%)	**31(43%)** [a]	47(65%)	25(35%)	32(44%)	40(56%)
Female	8(10)	8(100%)	0(0.0%)	5(63%)	3(37%)	3(37%)	5(63%)
Habit		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
Non-smoker	33(41)	28(85%)	5(15%)	24(73%)	9(27%)	14(42%)	19(58%)
Smoker	47(59)	21(45%)	**26(55%)** [b]	28(60%)	19(40%)	21(45%)	26(55%)
Tumor size		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
T1 (≤3cm)	32(40)	20(63%)	12(37%)	25(78%)	7(22%)	19(59%)	13(41%)
T2 (>3cm to ≤5cm)	17(21)	12(71%)	5(29%)	9(53%)	8(47%)	7(41%)	10(59%)
T3 (>5cm to ≤7cm)	11(14)	7(64%)	4(36%)	8(73%)	3(27%)	3(27%)	8(73%)
T4 (>7cm)	20(25)	10(50%)	10(50%)	10(50%)	10(50%)	6(30%)	14(70%)
Nodal status		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
N0	7(9)	4(57%)	3(43%)	4(57%)	3(43%)	2(29%)	5(71%)
N1	5(6)	4(80%)	1(20%)	5(100%)	0(0.0%)	3(60%)	2(40%)
N2	55(69)	39(71%)	16(29%)	35(64%)	20(36%)	23(42%)	32(58%)
N3	13(16)	2(15%)	**11(85%)** [c]	8(62%)	5(38%)	7(54%)	6(46%)
Stage		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
I	1(1)	0(0.0%)	1(100%)	0(0.0%)	1(100%)	0(0.0%)	1(100%)
II	5(6)	4(80%)	1(20%)	4(80%)	1(20%)	3(60%)	2(40%)
III	74(93)	45(61%)	29(39%)	48(65%)	26(35%)	32(43%)	42(57%)
Histological subtypes		49(61%)	31(39%)	52(65%)	28(35%)	35(44%)	45(56%)
Adenocarcinoma	40(50)	33(83%)	7(17%)	24(60%)	16(40%)	20(50%)	20(50%)
Squamous cell carcinoma	40(50)	16(40%)	**24(60%)** [d]	28(70%)	12(30%)	15(37%)	25(63%)

Note: p value: a$\chi 2$ =5.624, r= 0.265, p= 0.018, b $\chi 2$= 13.179, r= 0.406, p=0.0001, c $\chi 2$= 14.475, r= 0.215, p= 0.002, d $\chi 2$= 15.221, r= 0.436, p=0.0001

within the lung up to hilar nodes) (40% 2/45), lymph node involvement. With histological subtype higher abnormal LDH level was observed in squamous cell carcinoma (63%, 25/45) patients as compared to adenocarcinoma (50% 20/45) patients. (Table1a)

In SCLC, as all the patients were positive for p53 expression no correlation could be performed with clinical and pathological parameters higher BCL2 expression was observed in male patients (47%, 9/9) as only one female patient included in this study, and in smokers (50%, 7/9) compared to non-smokers (33% 2/9). Correlating BCL2 expression with pathological parameters, higher expression of BCL-2 was noted in patients with LN2 (nodes represent ipsilateral mediastinal or subcarinal lymphadenopathy) nodal status (50% 8/9) as compared to patients with LN3 (nodes represent contralateral mediastinal or contralateral hilar lymphadenopathy or scalene or supraclavicular nodes) nodal status (25%, 1/9). With disease stage, all the patients were of stage III disease and forty five (45%, 9/20) of patients showed positive BCL2 expression. A significant higher abnormal LDH level was noted in patients with younger age group ≤56 years of age (92%, 11/14; p=0.010), and a higher trend of abnormal LDH level was observed in T2 tumor size (88%, 7/8) followed by T4 (75%, 3/4), T1 (50%, 3/6) and T3 (50%, 1/2) tumors. As all the patients were of stage III disease and of them seventy (70%, 14/20) percent of patients had abnormal LDH level. (Table 1b)

Correlation of p53, BCL2 and Serum LDH with other diagnostic lung cancer panel

Further p53, BCL2 and Serum LDH expression in NSCLC were correlated with diagnostic lung cancer panel which included TTF-1(Thyroid transcription factor), CK7 (cytokeratin 7), CEA (carcinoembryonic antigen), p63 and CK5/6. In relation to p53 expression a significant inverse-correlation was noted with adenocarcinoma markers TTF –1 (p=0.003), CK7 (p=0.026) and CEA (p=0.033), with no significant correlation with squamous cell carcinoma markers. Also no significant correlation of

Table 1 (b): Correlation of BCL2 and Serum LDH with Clinical and pathological parameters in SCLC patients (N=20)

Parameters	N (%)	BCL-2 expression		LDH level	
	N (%)	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
Age(years)		11(55%)	9(45%)	6(30%)	14(70%)
≤56	12(60)	6(50%)	6(50%)	1(8%)	11(92%)a
>56	8(40)	5(63%)	3(37%)	5(63%)	3(37%)
Gender		11(55%)	9(45%)	6(30%)	14(70%)
Male	19(95)	10(53%)	9(47%)	6(32%)	13(68%)
Female	1(5)	1(100%)	0(0.0%)	0(0%)	1(100%)
Habit		11(55%)	9(45%)	6(30%)	14(70%)
Non-smoker	6(30)	4(67%)	2(33%)	2(33%)	4(67%)
Smoker	14(70)	7(50%)	7(50%)	4(29%)	10(71%)
Tumor size		11(55%)	9(45%)	6(30%)	14(70%)
T1 (≤3cm)	6(30)	4(67%)	2(33%)	3(50%)	3(50%)
T2 (>3cm to ≤5cm)	8(40)	3(37%)	5(63%)	1(12%)	7(88%)
T3 (>5cm to ≤7cm)	2(10)	2(100%)	0(0%)	1(50%)	1(50%)
T4 (>7cm)	4(20)	2(50%)	2(50%)	1(25%)	3(75%)
Nodal status		11(55%)	9(45%)	6(30%)	14(70%)
N0	0(0)	0(0%)		0(0%)	
N1					
N2	16(80)	8(50%)	8(50%)	5(31%)	11(69%)
N3	4(20)	3(75%)	1(25%)	1(25%)	3(75%)
Stage		11(55%)	9(45%)	6(30%)	14(70%)
I	0(0)	0(0%)		0(0%)	
II					
III	20(100)	11(55%)	9(45%)	6(30%)	14(70%)
Note: p value: a $\chi 2$ =6.706, r= -0.579, p= 0.010					

BCL2 expression and LDH level was noted with any of the adenocarcinoma and squamous cell carcinoma markers. (Table 2a)

In SCLC, besides NSCLC the diagnostic panel also included synaptophysin and chromogranin. In relation to BCL2 a significant positive correlation was noted with chromogranin, and higher trend was observed with synaptophysin. With LDH a significant inverse correlation of abnormal LDH was noted with CEA whereas, no significant correlation was noted with other markers. (Table 2b)

Univariate Survival Analysis

Disease free survival was not evaluated since majority of the patients had persistent disease. According to Kaplan-Meier univariate survival analysis, for overall survival (OS) a similar incidence of death was noted in patients with and without p53, BCL-2 expression A trend of higher incidence of death was noted in patients with abnormal LDH level as compared to patients with normal LDH level. (Table 3a)

However, Kaplan-Meier univariate survival analysis in adenocarcinoma patients, with respect to overall survival (OS) a higher incidence of death was noted in patients with positive p53 and BCL2 expression as compare to patients with negative expressions. In LDH a higher incidence of death was noted in patients with abnormal LDH level as compared to patients with normal LDH level. (Table 3b) In squamous cell carcinoma, a similar incidence of death was noted in patients with p53 positive and negative expression. A higher incidence of death was noted in patients with negative BCL-2 expression as compare to patients with positive BCL2 expression. In LDH a higher incidence of death was noted in patients with abnormal LDH level.(Table 3c)

In SCLC, since the patients had persistent disease, disease free survival was not evaluated. Out of 20 patients, 9 patients died and remaining 11patients were lost to follow-up with a median survival of 10 months. Hence, survival analysis was not evaluated. However, all the patients who died had abnormal LDH level.

Inter-marker correlation between p53, BCL2 and LDH

When intermarker correlation was performed among p53, BCL2 and abnormal LDH level, similar abnormal LDH levels were noted among patients with p53 positive (61%, 19/45) and p53 negative (53%,

Table 2 (a): Correlation of p53, BCL2 and Serum LDH with other diagnostic lung cancer panel (NSCLC)

Parameters	p53 expression		BCL-2 expression		LDH level	
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
TTF – 1	N=71		N=71		N=71	
	42(59%)	29(41%)	45(63%)	26(37%)	29(41%)	42(59%)
Negative	20(45%)	24(55%)	30(68%)	14(32%)	18(41%)	26(59%)
Positive	**22(82%)**[a]	5(18%)	15(56%)	12(44%)	11(41%)	16(59%)
CK7	N=71		N=71		N=71	
	41(58%)	30(42%)	46(65%)	25(35%)	31(44%)	40(56%)
Negative	10(40%)	15(60%)	18(72%)	7(28%)	9(36%)	16(64%)
Positive	**31(67%)**[b]	15(33%)	28(61%)	18(39%)	22(48%)	24(52%)
CEA	N=54		N=54		N=54	
	29(54%)	25(46%)	32(59%)	22(41%)	22(41%)	32(59%)
Negative	9(37%)	15(63%)	16(67%)	8(33%)	10(42%)	14(58%)
Positive	**20(67%)**[c]	10(33%)	16(53%)	14(47%)	12(40%)	18(60%)
P63	N=54		N=59		N=59	
	34(58%)	25(42%)	39(66%)	20(34%)	24(41%)	35(59%)
Negative	13(65%)	7(35%)	12(60%)	8(40%)	10(50%)	10(50%)
Positive	21(54%)	18(46%)	27(69%)	12(31%)	14(36%)	25(64%)
CK5/6	N=46		N=46		N=46	
	25(54%)	21(46%)	27(59%)	19(41%)	19(41%)	27(59%)
Negative	14(67%)	7(33%)	12(57%)	9(43%)	8(38%)	13(62%)
Positive	11(44%)	14(56%)	15(60%)	10(40%)	11(44%)	14(56%)
Note: p value: aχ2 =8.988, r= -0.356, p= 0.003, b χ2= 4.981, r= -0.265, p=0.026, c χ2= 4.562, r= -0.291, p= 0.033						

Table 2 (b): Correlation of BCL2 and Serum LDH with other diagnostic lung cancer panel (SCLC)

Marker	BCL2 expression		LDH level	
	Negative N (%)	Positive N (%)	Normal N (%)	Abnormal N (%)
TTF - 1	N=16		N=16	
	9(56%)	7(44%)	4(25%)	12(75%)
Negative	3(75%)	1(25%)	1(25%)	3(75%)
Positive	6(50%)	6(50%)	3(25%)	9(75%)
CK7	N=15		N=3	
	9(60%)	6(40%)	0(0%)	3(100%)
Negative	2(33%)	4(67%)	0(0%)	3(100%)
Positive	7(78%)	2(22%)	0(0%)	0(0%)
CEA	N=9		N=9	
	4(44%)	5(56%)	2(22%)	7(78%)
Negative	2(33%)	4(67%)	0(0%)	**6(100%)**[b]
Positive	2(67%)	1(33%)	2(67%)	1(33%)
P63	N=7		N=7	
	5(71%)	2(29%)	2(29%)	5(71%)
Negative	5(83%)	1(17%)	2(33%)	4(64%)
Positive	0(0%)	1(100%)	0(0%)	1(100%)
CK5/6	N=3		N=3	
	1(33%)	2(67%)	0(0%)	3(100%)
Negative	1(33%)	2(67%)	0(0%)	3(100%)
Positive	0(0%)	0(0%)	0(0%)	0(0%)
Synaptophysin	N=20		N=20	
	11(55%)	9(45%)	6(30%)	14(70%)
Negative	3(100%)	0(0%)	0(0%)	3(100%)
Positive	8(47%)	9(53%)	6(35%)	11(65%)
Chromogranin	N=20		N=20	
	11(55%)	9(45%)	6(30%)	14(70%)
Negative	**6(100%)**[a]	0(0%)	1(17%)	5(83%)
Positive	5(36%)	9(64%)	5(36%)	9(64%)
Note: p value: a χ2= 7.013, r= 0.592, p= 0.008, b χ2 =5.143, r= -0.756, p= 0.023				

26/45) expression. Similar BCL2 expression was observed among p53 positive (39%, 12/35) and p53 negative (33%, 16/35) tumors. Also, in correlation of BCL2 with abnormal LDH levels similar abnormal LDH level was noted in patients with BCL2 positive (61%, 17/45) and BCL2 negative (54%, 28/45) tumors. (Table 4)

Discussion

The present study evaluated 100 lung cancer patients in which, 80 patients of NSCLC (40 patients were of adenocarcinoma and 40 patients were of squamous cell carcinoma) and 20 patients of SCLC. In this study and in most of the studies majority of patients are presented with advanced disease at the time of diagnosis like stage III/IV.[11] So, despite of significant developments in the oncological management, the survival of late stage lung cancer over recent years remains poor.

The major causes which induce various types of cancers including lung cancer is altered metabolism which is further added as an emerging and potential hallmark of cancer and plays an important role in the cancer cell progression. LDH is considered relevant in all cancers due to its role as a metabolic check point in cancer glycolytic pathway and also plays a role in activation of some proto-oncogene and the maintenance of invasiveness and metastatic potential. Mutant p53 regulates various metabolisms by mediating metabolic changes such as alteration in morphology of gene and alteration of cellular metabolism that promotes tumor cell survival and growth.[8] Alteration in the expression and the function of

Table 3 (a): p53, BCL-2 and Serum LDH expression in relation to Overall survival. (NSCLC)

Markers Expression	N	OS in months Mean ± SE	Alive N (%)	Dead N (%)
p53 Expression				
Negative	49	21.33±2.12	21(43)	28 (57)
Positive	31	21.81±3.47	13 (42)	18 (58)
Log rank=0.03, df=1, p=0.957				
BCL-2 Expression				
Negative	52	19.99±2.16	22 (42)	30 (58)
Positive	28	23.86±3.19	12 (43)	16 (57)
Log rank=0.785, df=1, p=0.375				
LDH Level				
Normal	35	20.54±2.63	17 (49)	18 (51)
Abnormal	45	22.52±2.47	17 (38)	28 (62)
Log rank=0.540, df=1, p=0.462				

Table 3 (b): p53, BCL-2 and Serum LDH expression in relation to Overall survival in adenocarcinoma

Markers Expression	N	OS in months Mean ± SE	Alive N (%)	Dead N (%)
p53 Expression				
Negative	33	22.46±3.00	14 (42)	19 (58)
Positive	7	34.71±6.92	2 (29)	5 (71)
Log rank=0.997, df=1, p=0.318				
BCL-2 Expression				
Negative	24	21.86±3.48	11 (46)	13 (54)
Positive	16	27.62±5.88	5 (31)	11 (69)
Log rank=0.691, df=1, p=0.406				
LDH Level				
Normal	20	22.15±4.16	9 (45)	11 (55)
Abnormal	20	25.78±4.63	7 (35)	13 (65)
Log rank=0.819, df=1, p=0.366				

Table 3(c): p53, BCL-2 and Serum LDH expression in relation to Overall survival in Squamous cell carcinoma

Markers Expression	N	OS in months Mean ± SE	Alive N (%)	Dead N (%)
p53 Expression				
Negative	16	21.17±2.68	7(44)	9 (56)
Positive	24	19.19±3.29	11 (46)	13 (54)
Log rank=0.015, df=1, p=0.902				
BCL-2 Expression				
Negative	28	18.71±2.66	11 (39)	17 (61)
Positive	12	21.37±3.86	7 (58)	5 (42)
Log rank=0.319, df=1, p=0.572				
LDH Level				
Normal	15	18.89±3.36	8 (53)	7 (47)
Abnormal	25	21.04±2.91	10 (40)	15 (60)
Log rank=0.484, df=1, p=0.487				

Table 4: Intercorrelation between p53, BCL-2 and LDH

Parameters		LDH		BCL-2	
		Normal N=35(44%)	Abnormal N=45(56%)	Negative N=52(65%)	Positive N=28(35%)
p53	Positive (N=31)	12(39%)	19(61%)	19(61%)	12(39%)
	Negative (N=49)	23(47%)	26(53%)	33(67%)	16(33%)
		X2 = 0.522		X2 = 0.306	
		r = -0.081		r =0.062	
		P = 0.470		P = 0.580	
BCL-2	Positive (N=28)	11(39%)	17(61%)		
	Negative (N=52)	24(46%)	28(54%)		
		X2 = 0.349			
		r = -0.066			
		P = 0.555			

BCL2 contributes to the progression of human cancers. High BCL2 expression has been reported in many different tumors types including lung cancer, breast cancer and ovarian cancer.

p53 is frequently mutated in human tumors in the present study expression of p53 was seen in 39% of patients. The results were in accordance with Halvorsen et.al who observed p53 expression in 47% of patients and in discordance with the study of Mattioni et.al who observed p53 positive expression in 20% NSCLC patients which was lower compared to present study.[12-13] Over expression of p53 can induce circulating p53 antibodies in patients of various types of cancer, including lung cancer, because the altered conformation of p53 produced by mutations which may trigger an auto immune response once the protein has been released from tumor cells.[13-14] Cytoplasmic expression of BCL2 was found in 35% patients. In the study of Gryko et.al BCL2 positive expression was noted in 56% patients and which was higher compared to present study.[15] Fifty-six percent of patients showed abnormal LDH level, whereas 44% of patients showed normal range of LDH. (Normal range 100-190 IU/L) Similar to our study Lee et.al study showed 57% of patients with abnormal LDH level in lung cancer patients.[16]

Further, when p53 expression was correlated with the clinical parameters a significant higher p53 expression was seen in male patients, because majority of patients enrolled in the study were male. Also a significant higher p53 expression was observed in smokers. Our results indicate that smoking may play a critical role in promoting NSCLC progression via modulation of p53 protein expression which may be due to metabolic changes. As the risk of lung cancer increases with the age higher p53 expression was found in patients with >60 years of age which was in

accordance with the study of Xie et al.[17] In the study of Mattioni et al no significant difference with p53 expression was found with regard to age and gender.[13] In correlation of pathological parameters, a significant higher p53 expression was observed in patients with LN3 nodal status, similar to the study of Zhou et al that showed a significantly higher positive p53 expression in patients with lymph node metastasis.[18] When p53 expression was correlated with histological subtype, significant higher p53 expression was noted in patients with squamous cell carcinoma, suggesting that p53 mutations are usually gain before clonal expansion in SCC including those without histological evidence of precursor lesion.[19] Higher p53 expression was observed with advancement of disease stage and majority of patients enrolled in the study were of advance disease stage. However, study of Halvorsen et al showed that no significant correlation of p53 expression with tumor size and stage.[12]

Further BCL-2 expression when correlated with the clinical parameters higher BCL-2 expression was observed in smokers. No significant correlation was found between BCL-2 expression and gender or age as similar in the study of Anagnostou et al.[20] These results suggest that Bcl2 may be a primary target of carcinogens in tobacco smoke. With diseases stage, higher BCL-2 expression was noted in patient with stage III diseases as compared to patients with stage II. However, the study of Anagnostou et al showed no significant correlation of BCL-2 expression with stage.20 The study of Tsamandas et al observed that BCL2 expression in advanced-stage and high-grade gastric carcinomas indicate that BCL2 is involved in early stage of tumor development and might be playing a role in metabolic dysfunction.[21]

A higher LDH was noted in patients without lymph node involvement which was in discordance with study of Kayser et al which showed LDH level in correlation with lymph node metastasis revealed a statistically significant difference in regard to the intensity score between tumors with no or positive hilar lymph node metastases and mediastinal lymph node metastases.[22] In correlation with histological subtype of NSCLC, a higher expression of abnormal LDH level was found in patients with squamous cell carcinoma as compared to patients with adenocarcinoma. However, the study of Lee et al found that serum LDH levels were not significantly different according to histology.16 Higher LDH level was noted in patient with stage III diseases as compared to patients with stage II and there was only one patient with stage I diseases who showed abnormal LDH level. In advance disease stage induction of increased LDH level could be probably because of high amount of glucose uptake by cancer cells in hypoxic condition.

In SCLC, patients with ≤56 years of age show significant correlation with abnormal LDH which is discordance with the study of Lui et al who found that the LDH level was not related to clinical characteristics in SCC patients.[23]

Further p53 expression when correlated with lung cancer panel which showed a significant inverse-correlation with adenocarcinoma markers (Thyroid transcription factor) TTF-1, (cytokeratin 7) CK7 and CEA. The results were in discordance with study of Myong (2003)[24] who noted that there was no significant correlation between TTF-1 expression and over expression of p53. However, the study of Zhan et al study observed that TTF-1 over expression is associated with a favorable prognosis in patients with NSCLC.[25] whereas p53 over expression is associated with poor prognosis.24 No significant correlation of BCL-2 and Serum LDH was noted with adenocarcinoma markers TTF-1, CEA and CK7 as well as squamous cell carcinoma markers CK5/6 and p63.

A significant positive correlation of BCL-2 was noted with chromogranin, and a trend of higher expression was observed with synaptophysin. The study of Li et al, demonstrated that the expres¬sions of BCL-2 are more valuable than the highly spe¬cific markers such as synaptophysin, chromogranin.[26] With LDH a significant inverse correlation of abnormal LDH was noted with CEA. No significant correlation was noted with other markers.

No significant correlation was found between overall survival of patients and expression of p53, BCL-2 and abnormal serum LDH level. In this study when overall survival was correlated with respect to histological subtypes it was noted that patients with positive expression of p53, BCL-2 and Abnormal LDH and with adenocarcinoma histology shows increased incidence of death and poor survival.

In inter-marker correlation between p53, BCL-2 and sLDH showed that patients with higher p53 positive and BCL-2 positive expression had high LDH level. This result suggests that LDH might play an important role in apoptosis regulation. No significant correlation was noted between p53 and BCL-2. Similar results were obtained by the study of Yoo et al and suggested that there may be other oncogene products or additional factors that regulate apoptosis in vivo.[27]

Conclusion

In conclusion, higher expression of mutant p53 and anti-apoptotic protein BCL-2 in smokers suggests that higher tobacco consumption increased the risk of mortality or poor survival by inducing the altered metabolism of p53 and BCL-2 by effect of carcinogens. Higher p53 expression associated with squamous cell carcinoma.

A positive correlation of mutant p53 expression and BCL-2 expression with LDH in adenocarcinoma as well as impact of LDH on survival suggests that LDH plays an important role in cancer cell metabolism. The efficacy of drugs against LDH may be effective, however are under clinical trials.

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