Monaldi Archives for Chest Disease

eISSN 2532-5264 https://www.monaldi-archives.org/

Monaldi Arch Chest Dis 2025 [Online ahead of print]

To cite this Article:
Said AF, Mohamed HY, Kamaleldin AM, et al. **The diagnostic yield of the survivin gene in patients with lung cancer.** *Monaldi Arch Chest Dis* doi: 10.4081/monaldi.2025.3468

The diagnostic yield of the survivin gene in patients with lung cancer

Azza Farag Said,[1] Hager Yehia Mohamed,[1] Amel Mahmoud Kamaleldin,[2]
Shady Elia Anis,[3] Zainab Hassan Saeed[1]

[1]Chest Diseases Department, Faculty of Medicine, Minia University, Minia City; [2]Clinical Pathology Department, Faculty of Medicine, Minia University, Minia City; [3]Anatomic Pathology Department, Faculty of Medicine, Cairo University, Cairo City, Egypt

Correspondence: Azza Farag Said, Chest Diseases Department, Faculty of Medicine, Minia University, 32 Shalaby area, Minia City, Egypt.
Tel.: 01001862270. E-mail: azza.sayed@mu.edu.eg

Contributions: AFS, HYM, AMK, ZHS, conceptualization; HYM, data collection and analysis; AFS, ZHS, bronchoscopy technique; SEA, histopathology analysis; AMK, molecular assay aspect of the study. All authors contributed to the writing of the original draft preparation and editing, read and agreed to the published version of the manuscript. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this study.

Conflict of interest: the authors declare that they have no conflict of interest.

Ethics approval and consent to participate: all methods used in this study were done in accordance with the Declaration of Helsinki and clinical practice guidelines. The study protocol was approved by the Research Ethics Committee of Faculty of Medicine, Minia University, Egypt (21/11/2017).

Informed consent: an informed written consent was obtained from patients before enrollment.

Patient consent for publication: we took an informed written consent from patients for participating of the study and for publishing it.

Availability of data and materials: the datasets used during the current study are available from the corresponding author on reasonable request.

Funding: no funding was received for this study.

Acknowledgments: the authors would like to thank a lot for all the study participants.

Abstract

It has been discovered that many solid tumors express the survivin gene, particularly in tissue samples. On the other hand, limited data exist on the significance of the survivin gene in bronchial aspirates from lung cancer patients. The current study was designed to evaluate the levels of the survivin gene in lung cancer patients and correlate them with other clinical features. The study population consisted of 58 patients with lung cancer. A total of 25 patients with non-malignant lung diseases were used as a comparable group. We used real-time quantitative reverse transcription polymerase chain reaction to assess survivin gene level in bronchial aspirate during bronchoscopy from individuals with lung cancer, as well as those with benign lung diseases. Cases with lung cancer had bronchial aspirates with a substantially greater survivin gene level (3.7±1.8) compared to individuals with benign lung illnesses (1.1±0.9) (p=0.0001). The lung cancer diagnosis had a sensitivity of 74.14% and a specificity of 96% when 2.4 of the survivin gene was used as the cutoff value. The levels of the survivin gene in lung cancer patients were significantly positively correlated with both age and performance status, with p values of 0.012 and 0.0001, respectively. Nonetheless, there was a negative connection between the survivin gene level and the length of symptoms as well as the survival time in months, with p-values of 0.027 and 0.001, respectively. As a molecular marker, survivin gene identification in bronchial aspirate has both diagnostic and prognostic significance for lung cancer.

Key words: lung cancer, bronchial aspirate, survivin gene.

Introduction

Apoptosis is a natural mechanism of cellular death essential for the development and health of cells and tissues. Apoptosis pathway dysregulation causes a variety of diseases, including cancer, autoimmune and immunodeficiency diseases and neurological disorders [1].

A family of proteins called inhibitor of apoptosis proteins (IAPs) can halt the apoptosis process by directly binding to caspases. The baculoviral IAP repeat containing (BIRC) domain is found in all these IAP family members and spans around 70 amino acids. BIRC1 to BIRC8 are members of the IAP family that are encoded in the mammalian genome [2]. Various studies have discovered that IAP genes exhibit significant expression levels in different cancer cells and primary tumor biopsy samples [3].

Survivin, a diminutive IAP encoded by the baculoviral inhibitor of apoptosis repeat-Containing 5 (BIRC5) gene, was discovered throughout embryonic and fetal development. It is decreased and becomes unobservable in normal adult tissue, and was re-expressed in a variety of tumors, such as stomach, colon, ovary, lung, breast, pancreases, bladder and liver cancers [4].

Survivin gene (survivin mRNA) has sparked a lot of attention because of its potential relevance in cancer detection as a molecular diagnostic marker [5].

Lung cancer ranks as the foremost cause of death associated with cancer globally Bronchoscopic methods used to diagnose of suspected lung cancer involve the histological and cytological analysis of tissues from bronchial biopsy, bronchial wash (BW) and bronchoalveolar lavage (BAL). However, in about half of the cases, the first bronchoscopy does not yield a definitive diagnosis in patients suspected of lung cancer [6].

The use of molecular markers in standard cytology specimens is an approach to improving lung cancer diagnosis [7].

Most studies evaluating survivin gene expression in lung tissue samples. As a result, survivin gene as a molecular marker for the identification of primary lung cancer in pulmonary aspirate samples could be a promising tool, particularly in cases of peripheral lung malignancies where transbronchial biopsies are unable to provide a definitive diagnosis or where there is absence of endobronchial masses.

The current study aims to assess survivin gene mRNA expression in bronchial aspirate samples of patients with primary lung cancer as a diagnostic tool. In addition, correlation of survivin gene with other clinical characteristics was evaluated.

Materials and Methods

Study population

This prospective observational study enrolled 58 patients with primary lung cancer who were selected from Chest Diseases, Internal medicine and Oncology departments of Minia

University Hospital during the period of February 2018 to February 2020. Twenty-five patients with benign lung diseases were studied also as a comparable group;(10) patients with pneumonia , (8) patients with acute lung abscess , (5) patients with interstitial lung disease , and (2) patients with sarcoidosis

Suspicion of lung cancer was based on presence of abnormal chest X-ray findings suggestive of malignancy as pulmonary mass, nodule, area of consolidation with underlying collapse and confirmed based on histopathological examination. Patients with massive hemoptysis, metastatic lung cancer, contraindications to fiberoptic bronchoscopy and extra-pulmonary malignancies were excluded.

Clinical characteristics

Clinical data was recorded including demographics, smoking history, symptomatology, comorbidity and age-adjusted comorbidity index [8], hematological blood analysis including complete blood count, liver, renal function and coagulation profile. Computed tomography (CT) of the chest was done with IV contrast agent.

Bronchoscopy technique

Fiberoptic bronchoscopy (PENTAX, EB-1970(2.8mm) , Japan) was performed in Bronchoscopy unit of Chest Diseases Department at Cardiothoracic Minia University Hospital. Bronchoscopy was performed via the trans nasal or transoral route under local anesthesia (2% lidocaine spray) and conscious sedation in the form of intravenous midazolam (0.03-0.1 mg/kg). Transbronchial lung biopsy was taken in cases of endobronchial lesions for histological examinations and bronchial wash and or bronchoalveolar lavage (BAL) for cytologic study in cases of absent endobronchial lesions.

An endobronchial lesion yielded at least 5-6 tissue biopsy specimens. They were preserved in a 10% buffered formalin solution and paraffin embedding. Then the slides underwent hematoxylin and eosin staining before being examined histopathologically by a pathologist blinded to clinical data.

Bronchial wash (BW) was conducted by inserting a bronchoscope into an airway of an afflicted lung lobe and collecting specimens through the bronchoscope's suction channel after injecting 1-3 aliquots of 10 ml sterile normal saline into the working channel of bronchoscope. BAL was performed after inserting the bronchoscope into the tracheobronchial tree, the scope is directed into the subsegment of the lung to be examined based on the CT location of the lesion and advanced until the tip of the scope is wedged into a bronchiole. Using a handheld syringe, 20-50- ml of sterile normal saline at room temperature is injected and then gradually withdrawn back into the syringe. This procedure is done 3-5 times with a return sample yield

of 30% of the instillation deemed enough, with at least 10-20 ml necessary for cytological examination [9].

BAL was centrifuged to concentrate cells in fluid specimens onto a microscope slide which was then fixed in alcohol and stained with the Papanicalaou dye for evaluation of neoplastic cells. Without any knowledge of clinical data, BW and BAL results and biopsy slides were assessed independently.

Meanwhile, 25 patients with benign lung disease had their bronchial aspirate samples for cytologic examination.

Another amount of bronchial aspirate samples from all patients (bronchial wash in 53 patients and BAL in 30 patients) was used for survivin mRNA assay to which Ethylenediamine tetraacetic acid (EDTA) was added.

Staging of lung cancer was done according to TNM eight edition [10], and Eastern Cooperative Oncologic Group (ECOG) performance status was also studied [11].

Assessment of Survivin mRNA in bronchial aspirate

The expression of survivin mRNA (survivin gene) was done by utilizing Real- Time Quantitative Reverse Transcription polymerase chain reaction (qRT-PCR) with QuantiTect Reverse Transcription Kit (Qiagen, Germany). The assay was performed through 4 steps. First step was RNA extraction or purification using QIAamp RNA Mini Kits (Qiagen, Hilden, Germany) according to the Manufacturer's instructions. Briefly, the sample undergoes lysis under strongly denaturing conditions to deactivates RNases and guarantee the extraction of intact RNA. The buffering environment is subsequently modified to optimize the RNA adherence to the QIAamp membrane, after which the sample is applied to the QIAamp Mini spin column. The RNA adheres to the membrane while impurities are thoroughly removed through a two-step washing process with different wash buffers. RNA of superior quality is extracted in 30 µl of a specialized RNase-free buffer, making it immediately usable.

The second step is reverse transcription, where RNA was converted into complementary DNA (cDNA) utilizing QuantiTect Reverse Transcription Kit provided from Qiagen (Germany). The third step involves using cDNA from all samples as a template for exponential amplification by RT-PCR assay, performed on a DTlite Real-Time PCR System (DNA technology, Moscow, Russia). The reaction employed primers specific to cDNA for amplification and was labeled with SYBR Green dye. SYBR Green dye generated a fluorescent signal upon binding to the double-stranded DNA in solution. The cDNA quantity can be compared to that of reference gene, Glyceraldehydes 3- phosphate dehydrogenase (GAPDH), by using the amount of housekeeping gene sequence.

In quantitative PCR, DNA amplification is tracked at all PCR cycles. During the log linear phase of amplification, fluorescence level rises above the background. The stage where fluorescence becomes detectable is referred to as threshold cycle (Ct) or the Quantification Cycle (C_q). Real – time PCR DT master software provides Ct as a numerical parameter.

Last step is the quantification of survivin gene (mRNA) transcripts; in SYBR Green PCR kit to measure target DNA utilizing comparative Ct (ΔCt) quantification techniques to determine the ratio between the amount of target DNA relative to a sample called calibrator. A calibrator, which is a control with a specified analyte concentration, is utilized to confirm that an assay is operating correctly. The ΔCt of each experimental sample was subtracted from the ΔCt of the calibrator. The normalized quantity of survivin (linear value), relative to the reference (GAPDH) and the calibrator, was calculated by assessing the expression of 2-ΔΔCT.The thermal profile applied was as follows: one cycle for 15 min at 95°C for initial activation,15 seconds at 94°C for denaturation, followed by 40 cycles of amplification , with each cycle consisting of 20 seconds at 55°C and 20 seconds at 72°C each (annealing-extension step).

-Patient follow-up was obtained over the phone with the study population. Follow-up took place from the time of diagnosis and for three years or until someone passed away.

Statistical analysis

The statistical package of social science (SPSS), version 20 for Windows, was used for all of the analyses. Chi-square and Z tests were used to compare qualitative data that were reported as numbers and percentages. The statistical numerical data were presented as range and means ± standard deviation (SD). To compare two independent groups of quantitative data that was normally distributed, the student t-test was employed. The Mann-Whitney test was employed to compare non-parametric data between two independent groups. The Kruskal-Wallis test was used for non-parametric data and the one-way ANOVA test for normally distributed data when comparing more than two independent groups. $P < 0.05$ was the threshold for statistical significance. In order to determine the appropriate cutoff values with the highest sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy for the variables under test, receiver operating characteristic (ROC) curves were developed. For correlations, the non-parametric Spearman test was used. The association between survivin gene level and the survival time of lung cancer patients was assessed using Kaplan-meier survival curves.

Results

On studying the general features among the studied patients, it was found that there was no difference in the mean age in patients with lung cancer and those with benign lung diseases

(58.9 ± 10.7) and (55.9 ± 8.8) respectively ($p > 0.05$). Also smoking history had no significant difference ($p > 0.05$). While, analysis of gender distribution, male sex was significant in lung cancer cases (84.5%, p=0.038) and females were more among benign disorders (36%, p=0.02). Considering comorbidity, 87% of lung cancer patients had more than one comorbidity (p=0.001) , on the other hand, about 50% of patients with non-malignant diseases had no comorbidity(p=0.003). The Age adjusted Charlson comorbidity index (ACCI) score > 6 was more in patients with lung cancer in comparison with those of benign diseases (65.5% vs 4%, respectively, p=0.0001) (Table 1).

Figure 1 shows survivin gene levels (quantity) among the studied cases. It was found that patients with lung cancer had a mean value of 3.7±1.8 (range: 1.1-11.6) which was higher than the mean value of cases with benign pulmonary diseases (1.1±0.9) with a range of 0.1-2.7.

Of the cases of lung cancer, 12 patients had mucosal alterations from which bronchoscopic samples were obtained, revealing malignant cells, and 41 patients had endobronchial lesions that were positive to be malignant on histological examination. Using CT guided transthoracic biopsy, lung carcinoma was identified in the remaining 5 patients. The histological examination results led to the division of the patients into two groups: 10 with small cell lung cancer (SCLC) and 48 with non- small cell lung cancer (NSCLC). The NSCLC group consisted of twenty individuals diagnosed with adenocarcinoma, twelve with large cell carcinoma fourteen with squamous cell carcinoma, and two with adenosquamous carcinoma.

The ROC curve analysis reveals the performance of survivin gene in diagnosing lung cancer; the AUC was 0.929 (95% CI 0.845-0.971) at a cut-off value of 2.4 with a sensitivity and specificity of 74.14% (95% CI 61- 84.7) and 96% (95% CI 76.9-99.9) respectively (Figure 2).

Table 2 shows that 8.6% of lung cancer patients were at stage I, 34.5% at stage II, 20.7% at stage III and 36.2% at stage IV with the level of survivin gene increased significantly with advanced stage of lung cancer(p=0.001). Alternatively, survivin gene levels was not statistically significant in patients with NSCLC vs those of SCLC (p=0.945).It was found that dead patients of lung cancer had a significant higher level of survivin gene than alive patients (4.2 ±1.6 vs 1.8± 0.5 respectively, p=0.001).

Regarding correlation of survivin gene levels and some clinical data of lung cancer patients, there was a significant positive correlation with age and performance status of the patients. In contrast, there was a significantly negative correlation with the duration of illness and survival time (Table 3).

On analysis of Kaplan Meier curve of survival in relation to survivin gene level (Figure 3), at the 10-month mark, 100% of patients with survivin gene levels less than 2.4 and 70% of patients with levels greater than 2.4 were still alive. Thirty percent and 10% of patients with

survivin gene levels less than 2.4 and greater than 2.4, respectively, were still alive after a 30-month follow-up period. Those with survivin gene levels less than 2.4 exhibited a substantially extended survival time compared to those with survivin gene levels greater than 2.4 (p=0.045).

Discussion

An imbalance between cell proliferation and death is a component of the process that leads to lung cancer carcinogenesis. When cell apoptosis is prevented, abnormal tumor cell proliferation may arise, which ultimately provides support for carcinogenesis, development, invasion, and metastasis [12]. Numerous apoptotic biomarkers can be detected in blood and tissues like activated caspases 2,3,7,8 and 9, Apo-1/Fas, Fas ligand(sFasL), p53, phospo-p53, p21 ^{wafi},pH2AX, Bc1-2/Bc1-x1/McI-1, cytochrome C, Externalised phosphatidylserine and nucleosomal DNA [13]. In order to provide a molecular marker to diagnose lung cancer, we set out to assess the sensitivity and specificity of the survivin gene, one of the most significant IAP family inhibitors, in bronchial aspirates among patients with lung cancer.

Our study's findings demonstrated that, in bronchial aspirates from lung cancer patients, survivin gene levels were considerably greater than those from individuals with benign lung illnesses (3.7 ± 1.8 vs. 1.1 ± 0.9, p=0.0001). Li et al.'s study [14], which included 70 lung cancer patients and 26 patients with benign lung diseases, was consistent with our research in that they found that patients with lung cancer exhibited significantly increased levels of the survivin gene from their bronchial aspirates than patients with benign lung diseases (0.442 ± 0.264 vs. 0.195 ± 0.082, $p < 0.001$). Another study by Falleni et al. [15] examined at 10 non-neoplastic lung tissue samples and 83 NSCLC tissue samples. They discovered that 80 carcinomas (96%) had higher survivin gene levels than normal lung tissue samples ($p = 0.008$). On ROC curve analysis, which resulted in a diagnostic accuracy (area under the ROC curve) for survivin gene of 0.929 (95% CI 0.845-0.971). After determining the optimal cutoff value, the diagnostic utility of survivin gene detection in bronchial aspirates among patients with lung cancer was assessed regarding sensitivity, specificity, and predictive value. Survivin gene sensitivity was 74.14% (95% CI 61-84.7) and specificity was 96% (95% CI 76.9-99.9) with positive and negative predictive values of 97.7 and 61.5%, respectively, when 2.4 of survivin gene was used as the cutoff value.

According to Li et al. [14], survivin gene expression in bronchial aspirates exhibited 83 and 96% sensitivity and specificity respectively for lung cancer diagnosis. Survivin gene levels in blood have sensitivity and specificity of 23.7 and 90% for lung cancer diagnosis, according to research by Xiu et al. [16], and 71.4 and 100% for lung cancer diagnosis according to Hassan et al study [17].

About survivin gene level in different TNM stages, in the current study, we identified a

significant difference between survivin gene level and various stages of lung cancer. Since early stages (I&II) of lung cancer (2.3 ± 0.9, 2.5 ± 1.1) exhibited a significantly lower survivin gene level than later stages (III&IV) (4.1 ± 1, 4.9 ± 1.9) (p=0.001).

The meta-analysis verified that NSCLC patients at TNM III/IV stage exhibited considerably higher survivin gene level than those at TNM I/II stage (P < 0.001) [18]. Another research examined the tumour sections of thirty-two individuals with SCLC, they found a significant difference in survivin gene level between the I&II and III&IV stages, with advanced stages exhibiting higher survivin gene level (P = 0.012) [19]. Two additional studies, however, did not discover any appreciable variations between TNM stage and survivin gene level. According to one of them, the Falleni et al. study [15], this discrepancy might be explained by the fact that only stage I (IA and IB) cancers are involved. The difference in the second study, Li et al. study [14], could be the result of racial variance and the use of different kits.

Regarding the histological subtype of NSCLC, we observed that large and squamous cell carcinoma had significantly higher survivin gene levels than adenocarcinoma (5.6 ± 2.3, 3.8 ± 0.7 versus 2.4 ± 1.1) (p=0.001). Falleni and colleagues [15] discovered that squamous cell carcinoma had a much greater survivin gene level than other tumour types (p=0.002), which is consistent with these results.

Regarding correlation of survivin gene level in bronchial aspirates with other clinical variables in patients with lung cancer, it was found that there was a significant positive association between age of the patients and survivin gene level (r=0.32, p=0.012). Previous study have shown that age-related accumulation of survivin gene can offers novel vision [20]. It offers fresh perspectives on the molecular processes underlying the connection between cancer, apoptosis, and ageing.

Among lung cancer patients, it was shown that those with lower performance status had a significantly lower survivin gene level than those with higher scores (r=0.76, p=0.0001). However, upon analyzing tissue samples from 60 NSCLC patients, one study was unable to detect any correlation between survivin gene level and performance status [21]. It might be because they only included patients with 0&1 performance status among NSCLC patients, but the current one included all performance status scores and included all forms of lung cancer.

Additionally, a significant negative connection (r= -0.48, p=0.001) was found in this study between survivin gene level and survival time and this was confirmed also of survival curve analysis (Figure 3) , which are consistent with earlier research [22],they found that overall survival was considerably lower among NSCLC patients with positive nuclear survivin gene staining. Karczmarek-Borowska and colleagues demonstrated that patients exhibiting overexpression of survivin gene had a five- year survival was 14.3%, whereas those without gene expression had a significantly higher survival rate of 60% (p = 0.0003) [21]. On the other

hand, survivin gene level and survival outcome did not significantly correlate, according to earlier study [15]. This discrepancy could be attributed to the selection of NSCLC patients with early stage I, whereas our analysis covered lung cancer in all stages.

The current study has some limitation, the limited sample size of lung cancer patients is one of them, which could compromise its validity. Second, the study was conducted at a single center, which restricts how broadly the results may be applied. Furthermore, a single survivin gene evaluation at diagnosis would not fully reflect the dynamic relationship with therapy response or illness progression. Finally, it was preferable to concurrently evaluate the survivn gene on NSCLC tissue samples in order to confirm our findings.

Conclusions

Survivin gene can be found in bronchial aspirates with a fair sensitivity and specificity, making it a viable molecular marker for lung cancer identification. In addition to its diagnostic value, it has a prognostic issue. It is advised to conduct further prospective studies with a larger sample size to improve the validation of the findings.

References

1. Su Z, Yang Z, Xu Y, et al. Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol Cancer 2015;14:48.

2. Mobahat M, Narendran A, Riabowol K. Survivin as a preferential target for cancer therapy. Int J Mol Sci 2014; 15: 2494-516.

3. Tamm I, Kornblau SM, Segall H, et al. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. Clin Cancer Res 2000;6:1796-803.

4. Altieri DC. The molecular basis and potential role of survivin in cancer diagnosis and therapy. Trends Mol Med 2001;7:542-7.

5. Wu YK, Chen KT, Kuo YB, et al. Quantitative detection of survivin in malignant pleural effusion for the diagnosis and prognosis of lung cancer. Cancer Lett 2009;273:331-5.

6. Roth K, Hardie JA, Andreassen AH, et al. Predictors of diagnostic yield in bronchoscopy: a retrospective cohort study comparing different combinations of sampling techniques. BMC Pulm Med 2008;8:2.

7. Hirsch FR, Merrick DT, Franklin WA. Role of biomarker for early detection of lung cancer and chemoprevention. Eur Respir J 2009;19:1151-8.

8. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47:1245-51.

9. Du Rand IA, Blaikley J, Booton R, et al. British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. Thorax 2013;68:i1-44.

10. Rami-Porta R, Bolejack V, Crowley J, et al. The IASLC lung cancer staging project: proposals for the revisions of the T descriptors in the forthcoming eighth edition of the TNM classification for lung cancer. J Thorac Oncol 2015;10:990-1003.
11. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-55.
12. Hengartner MO. The biochemistry of apoptosis. Nature 2000;407:770-6.
13. Ward TH, Cummings J, Dean E, et al. Biomarkers of apoptosis. Br J Cancer 2008;99:841-6.
14. Li J, Chen P, Li XQ, et al. Elevated levels of survivin and livin mRNA in bronchial aspirates as markers to support the diagnosis of lung cancer. Int J Cancer 2013;132:1098-104.
15. Falleni M, Pellegrini C, Marchetti A, et al. Survivin gene expression in early-stage non-small cell lung cancer. J Pathol 2003;200:620-6.
16. Xiu Y, Sun B, Jiang Y, et al. Diagnostic value of the survivin autoantibody in four types of malignancies. Genet Test Mol Biomarkers 2018;22:384-9.
17. Hassan MH, Abuhamdah S, Abdel-Bary M, et al. Circulating and local nuclear expression of surviving and fibulin-3 genes in discriminating benign from malignant respiratory diseases: correlation analysis. Biosci Rep 2021;41:BSR20203097.
18. Duan L, Hu X, Jin Y, et al . Survivin protein expression is involved in the progression of non-small cell lung cancer in Asians: a meta-analysis. BMC Cancer 2016;16:276.
19. Yano, Y, Otsuka T, Hirano H, et al. Nuclear survivin expression in small cell lung cancer. Anticancer Res 2015;35:2935-9.
20. Al-khalaf HH , Aboussekhra A . Survivin expression increases during aging and enhances the resistance of aged human fibroblasts to gentoxic stress. Age 2013;35:549-62.
21. Karczmarek-Borowska B, Filip A, Wojcierowski J, et al. Survivin antiapoptotic gene expression as a prognostic factor in non-small cell lung cancer: in situ hybridization study. Folia Histochem Cytobiol 2005;43:237-42.
22. Lu B, Gonzalez A, Massion P, et al. Nuclear survivin as a biomarker for non-small-cell lung cancer. Br J Cancer 2004;91:537-40.

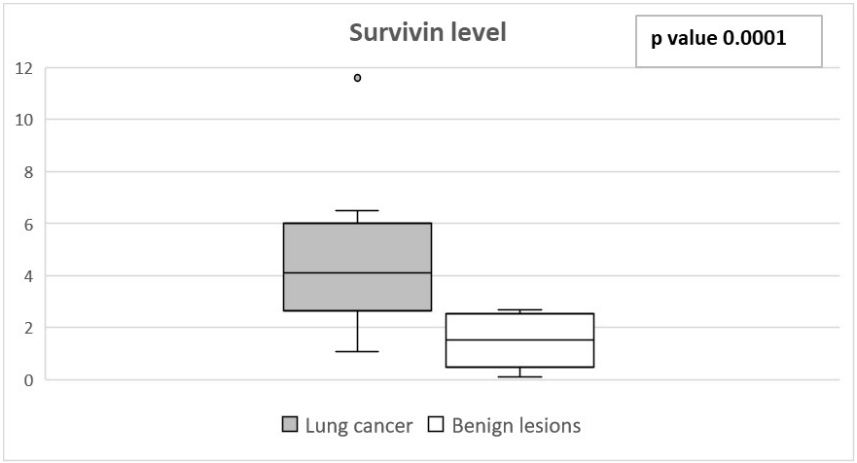

Figure 1. Survivin gene level among the studied patients.

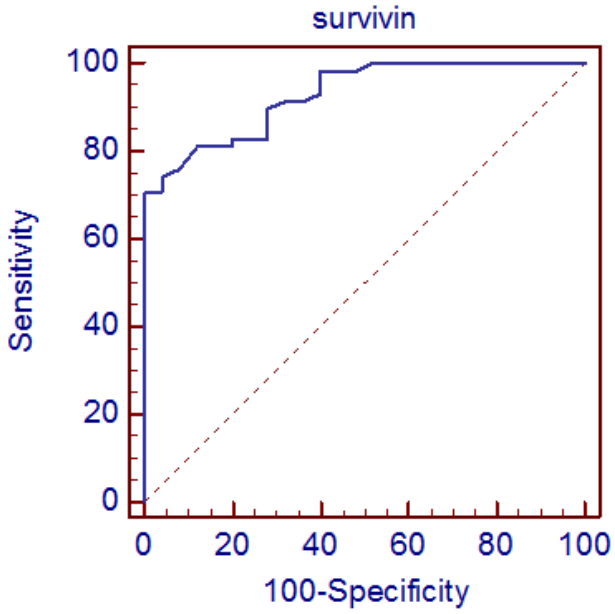

Figure 2. ROC curve analysis of survivin gene level in relation to lung cancer diagnosis.

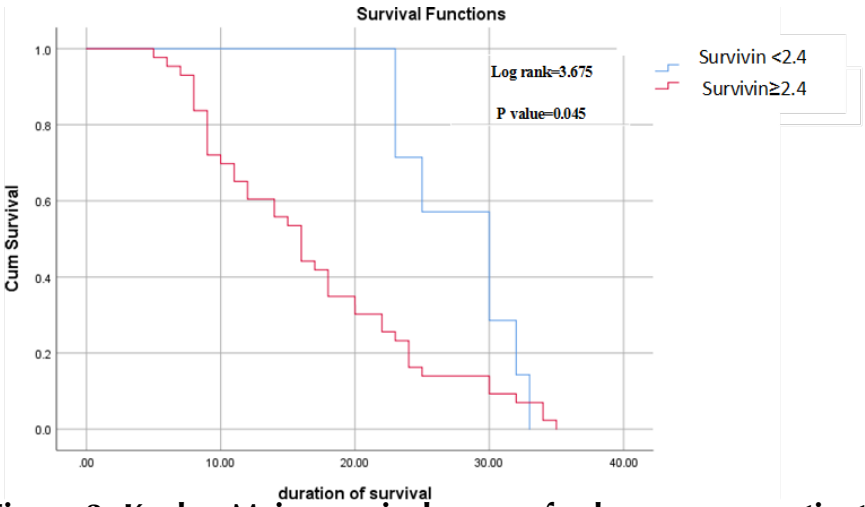

Figure 3. Kaplan Meier survival curves for lung cancer patients in relation to survivin gene level.

Table 1. Demographic data among the studied patients.

Variable	Lung cancer (n=58)	Benign diseases (n=25)	p-value
Age			
Range	32-80	38-71	
Mean±SD	58.9 ± 10.7	55.9 ± 8.8	0.224
Gender			
Males	49 (84.5%)	16 (64%)	**0.038**
Females	9 (15.5%)	9 (36%)	**0.02**
Smoking status			
Non-smoker	6 (10.3%)	6 (24%)	0.105
Current smoker	20 (34.5%)	7 (28%)	0.562
Ex-smoker	24 (41.4%)	7 (28%)	0.246
Passive smoker	8 (13.8%)	5 (20%)	0.477
Comorbidity			
No comorbidity	7 (12.1%)	12 (48%)	**0.003**
1 comorbidity	51 (87.9%)	13 (52%)	**0.001**
Age adjusted Charlson comorbidity index (ACCI) score			
0-1	0	8 (32%)	**--**
2-3	8 (13.8%)	10(40%)	**0.008**
4-5	12 (20.7%)	6 (24%)	0.727
>6	38 (65.5%)	1 (4%)	**0.0001**

Data is presented as numbers and percentages, Z test and independent t tests are used.

Table 2. Survivin gene levels in relation to clinical variables

TNM staging	**Survivin gene level Mean ± SD**	**p-value**
Stage I (no=5)	2.3±0.9	**0.001**
Stage II (no=20)	2.5±1.1	
Stage III (no= 12)	4.1±1	
Stage IV (no= 21)	4.9±1.9	
Histopathological type		0.945
NSCLC (no=48)	3.9±2.1	
SCLC (no=10)	3.7±1	
Survival		**0.001**
Alive (no=12)	1.8±0.5	
Dead (no=46)	4.2±1.6	

NSCLC, non small cell lung cancer; SCLC, small cell lung cancer. Kruskal-Wallis test is used among the different TNM stages of lung cancer.

Table 3. Correlation coefficient between survivin gene level and clinical data among lung cancer patients.

Variable	**r**	**p-value**
Age	0.32	0.012
Duration of illness	-0.29	0.027
Performance status	0.76	0.0001
Survival time	-0.48	0.001