Original Article

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Prognostic and Predictive Value of Permeability-Glycoprotein, Ki-67, and Interleukin-6 in Lung Cancer Patients

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Abstract:

Objective: Lung cancer patients often present with metastatic disease at the time of diagnosis, which affects their treatment regimen. Therefore, early diagnosis is crucial for improving patient survival. This study aimed to evaluate the roles of P-glycoprotein (P-gp), Ki-67, and interleukin-6 (IL-6) as diagnostic and predictive indicators in lung cancer. A secondary aim was to assess the prevalence of multi-drug resistance associated with P-gp gene expression in these patients.

Material and Methods: This study included 90 lung cancer patients, consisting of 60 with non-small cell lung cancer (NSCLC) and 30 with small cell lung cancer (SCLC). Additionally, 75 control samples were matched for age, gender, and smoking status. The levels of P-gp, Ki-67, and IL-6 in serum were determined using enzyme-linked immunosorbent assay (ELISA). P-gp gene expression was assessed via reverse transcription-polymerase chain reaction (RT-PCR).

Results: The results indicated that P-gp levels were significantly elevated in SCLC and NSCLC patients (10.35±0.51 ng/ml and 10.06±0.32 ng/ml, respectively) compared to the control group (3.79±0.21 ng/ml; p-value=0.0001). Ki-67 and IL-6 levels also significantly increased in SCLC patients (15.28±0.27 ng/ml and 54.46±2.11 ng/ml, respectively) compared to NSCLC (13.97±0.52 ng/ml and 46.17±1.18 ng/ml, respectively) and the control group (8.43±0.24 ng/ml and 26.40±1.01 ng/ml, respectively; p-value=0.0001). Additionally, mRNA expression of P-gp was significantly higher in lung cancer patients compared to controls.

Conclusion: Elevated levels of P-gp, Ki-67, and IL-6 may serve as useful diagnostic and predictive markers for lung cancer. Moreover, increased mRNA expression of P-gp suggests that lung cancer patients may exhibit resistance to chemotherapy.

Keywords: lung cancer, multiple drug resistance, non-small cell lung cancer, P-glycoprotein, prognostic

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Introduction

Lung cancer is one of the leading causes of cancer-related mortality globally, with approximately 1.2 million new cases identified each year[1]. It is estimated that 80–85% of lung cancer cases are non-small cell carcinomas (NSCLC)[2]. Early-stage lung cancer can potentially be treated with various options, including surgery, radiotherapy, and chemotherapy; however, advanced or metastatic lung cancer is usually incurable[1]. Despite advancements in treatment, the overall 5-year survival rate remains only 16%[3]. Measuring immune tumor markers is beneficial for the early detection of lung cancer, serving as a supplementary tool to clinical and other diagnostic tests for identifying cancer progression and monitoring treatment efficacy[4]. In this study, the immune tumor markers evaluated were P-glycoprotein, Ki-67, and IL-6.

The development of the multidrug resistance (MDR) phenotype is a significant obstacle in lung cancer therapy[5]. One common mechanism by which resistance to anticancer agents develops is the reduction of intracellular drug accumulation, often due to the expulsion of the drug through multidrug resistance–associated proteins (MRPs), such as P-glycoprotein[6]. High levels of P-gp are expressed by cancer cells, enhancing resistance to multiple drugs[7]. Inhibiting P-gp may increase the sensitivity of lung cancer cells to chemotherapy drugs[6,7]. Identifying the multidrug resistance phenotype could prevent unnecessary chemotherapy in cases where lung tumors are likely to be resistant to treatment. The predictive value of P-gp as an indicator of clinical chemotherapy resistance warrants further investigation[8].

Ki-67 is a DNA-binding nuclear non-histone protein expressed throughout the cell cycle[9]. It plays a crucial role in controlling and timing cell proliferation[10]. Ki-67 indicates the cell growth ratio and is considered a potent biomarker with significant prognostic and predictive value in major cancer types, including breast, prostate, lung, and colorectal cancer[11]. In malignant tumors, the percentage of Ki-67 positive cells is often linked to tumor aggressiveness and metastasis, highlighting its practical role in tumor histopathology. Consequently, the proliferation index, determined by Ki-67 levels, influences clinical decision-making and treatment choices across various tumor types[12]. Furthermore, numerous studies suggest that high Ki-67 expression serves as a strong prognostic factor in lung cancer[11].

IL-6 is a pro-inflammatory cytokine that plays a central role in the host defense mechanism by regulating immune responses and acute phase reactions[13]. In lung cancer, IL-6 is implicated in stimulating tumor cell proliferation, malignant transformation, and tumor progression. Previous studies have shown that elevated levels of circulating IL-6 are associated with shorter survival in patients with renal cell carcinoma, chronic lymphocytic leukemia, and lung cancer[14]. Additionally, IL-6 expression correlates with poor prognosis in lung cancer patients[15]. Chemotherapeutic agents have been shown to induce IL-6 production[16]; thus, it is vital to determine whether IL-6 levels can serve as independent prognostic factors for lung cancer survival, particularly in patients treated with chemotherapy[14].

Therefore, the primary purpose of this study was to evaluate the roles of P-gp, Ki-67, and IL-6 as diagnostic and predictive indicators in lung cancer. The secondary aim was to estimate the prevalence of multidrug resistance associated with P-gp gene expression in these patients.

Material and Methods

Study design

This study involved lung cancer patients from the Anbar Cancer Center, in Al-Ramadi City: from December 2022 until December 2023. This was a cross-sectional study, including 90 lung cancer patients (52 males and 38 females), with a mean age of 65.2 years. These patients included: 53.3% smokers and 46.6% non-smokers. The lung cancer patients were divided into 60 non-small cell

lung cancer patients and 30 small cell lung cancer patients. A pulmonologist, pathologist, and an oncologist evaluated the diagnosis of lung cancer patients at different stages: I, II, III and IV. For comparison, 75 samples were included in a control group (43 males and 32 females), with a mean age of 65.16 years. The control group included: 53.3% smokers and 46.6% non-smokers.

Approval for the study was obtained from the local Research Ethics Committee, of the University of Anbar/ College of Medicine (Approval No: 124 on November 23, 2023), in accordance with the Helsinki Declaration for Human Studies.

Sample selection

Inclusion criteria for patients required that they were newly diagnosed with lung cancer and had not initiated any treatment or received chemotherapy (cisplatin, etoposide or carboplatin) for less than two months. While, patients having had a family history of cancer, history of another kind of malignancy, or co-morbidities, such as being HBsAg positive, were excluded.

Control inclusion criteria specified individuals in good health without endocrine problems, hypertension, diabetes, compromised immune systems, acute infections or illnesses.

Samples collection and laboratory measurements

Prior to blood collection, patients and controls underwent an interview to obtain characteristic data, including gender, age, duration of lung cancer, height/ weight and smoking status. Written informed consent was obtained from each participant before participation in this study. Blood samples of five milliliters were drawn from each individual after a 12-hour fast. The blood was divided into two portions: 0.5 mL was added to a tube containing TRIzol™ Reagent for gene expression diagnostics, using Real-Time polymerase chain reaction (RT-PCR), and 4 mL was placed in plain tubes, allowed to clot, and then centrifuged for ten minutes at 3000 rounds per minute to obtain sera. The sera were then dispensed into three Eppendroff tubes, and the tubes were kept at -20 ˚C until the P-GP, Ki-67, and IL-6 levels were further analyzed using ELISA.

Determination of P-Glycoprotein, Ki-67, and interleukin-6 by enzyme-linked immunosorbent assay (ELISA)

The samples were evaluated using ELISA assays with kits for P-gp (Cat. No: ELK2148), Human Ki-67 (Cat. No: E-EL-H5432), and Interleukin-6 (Cat. No: ELK1156); all supplied by the ELK Biotechnology Company in China. The assays were conducted following the manufacturer's instructions, and the sample color was measured with an ELISA reader at 450±10 nm. The levels of these markers in each sample were determined by comparing their optical density (OD) to a standard curve.

Detection of P-GP or multidrug resistance 1 (MDR1) gene expression by Real-time PC:

For RNA analyses, each 0.5 mL of blood was added to 0.5 mL of TRIzol™ Reagent. Total RNA was isolated from blood samples; according to the protocol of TRIzol™ Reagent. Then, RNA yield was determined by a Quantus Fluorometer. This was employed to measure the extracted RNA concentration in order to detect the sample quality for usage in subsequent applications. Afterward, 200 µl of diluted QuantiFlour Dye was combined with 1 µl of Ribonucleic acid (RNA). RNA concentration values were detected during a 5-minute incubation period, in a dark place at room temperature. Using oligo (dT) primer, first-strand complementary deoxyribonucleic acid (cDNA) was synthesized from the RNA: lyophilized primers were provided by the Macrogen Company. Nuclease-free water was used to dissolve lyophilized primers, resulting in a stock solution with a prepared final concentration of 100 pmol/µl. To create a usable primer solution at 10 pmol/µl, 10 µl of the

primer stock solution (stored at -20 °C in the freezer) was mixed with 90µl of nuclease-free water. cDNA (1ng/µl) was then amplified in 9 µl PCR reaction solutions containing a 0.25 mmol/l MgCl2, 5 µl qPCR Master Mix, 0.5 µM Forward and 0.5 µM reverse primers, 2.5 µl Nuclease Free Water, and a 0.25 µl RT mix. The program of RT-PCR started with one cycle of RT. enzyme activation at 37 °C for 15 min. Initial denaturation was at 95 °C for 5 min, followed by 55 cycle denaturation at 95 °C for 20 s; annealing at 60 °C or 65 °C for 20 s, and extension at 72 °C for 20 s, so as to amplify product sizes for the amplification of cDNA of interest.

Table 1 Design of the MDR1 gene and β-Globin (Housekeeping gene) primers

Gene	Primer	Sequence	Annealing temp. (°C)
β-Globin	Forward primer	5'- ACACAACTGTGTTCACTAGC -3'	65
	Reverse primer	5'- CAACTTCATCCACGTTCACC -3'	
MDR1_exp	Forward primer	5'- GCTGTCAAGGAAGCCAATGCCT -3'	60
	Reverse primer	5'- TGCAATGGCGATCCTCTGCTTC -3'	

MDR1=multidrug resistance 1

Analysis Expression of Gene, using Livak Method: Relative quantification[17]:

$$\text{Folding} = 2^{-\Delta\Delta CT}$$

$$\Delta CT = CT_{\text{MDR1 gene}} - CT_{\text{House Keeping gene}}$$

$$\Delta\Delta CT = \Delta CT_{\text{patients}} - \Delta CT_{\text{Control}}$$

Statistical Analysis

The effect of variance variables on study parameters was determined using the SAS (2018) program. To statistically compare means, the T-test and the least significant difference (LSD) test (ANOVA) were employed. The chi-square test was used to evaluate the various types of lung cancer at p-value<0.05 and 0.01. The diagnostic specificity, sensitivity, and predictive value of P-gp, Ki-67, and IL-6 were estimated using the ROC (Receiver Operating Characteristic) curve. ROC curve analysis helped determine the ideal diagnostic cut-off values for maximizing clinical specificity and sensitivity. Progression-free survival (PFS) was analyzed for all indicator cutoff values using the Kaplan-Meier method, complemented by a log-rank test to assess significance.

Results

Indicated clinical characteristics of patients and control

The findings indicated similarities in age, gender, and BMI between patients and the control group. There were no significant differences in the mean age between lung cancer patients (65.2±0.82 years) and the control group (65.16±0.60 years). The average BMI of lung cancer patients (25.4±0.23 kg/m^2) was nearly equal to that of the control group (25.5±0.22 kg/m^2). The percentage of males in both lung cancer (57.7%) and control groups (57.4%) was higher than that of females (42.2% in lung cancer and 42.5% in control). Among the 90 serum samples from lung cancer patients, 30 had small cell lung cancer (SCLC) and 60 had non-small cell lung cancer (NSCLC). Using the TNM staging system, lung cancer patients were categorized into four stages: Stage IV had the highest percentage (34.4%),

followed by Stage III (27.7%), while Stages I and II had lower percentages (16.6% and 21.1%, respectively), as illustrated in Table 2.

Determination of P-glycoprotein, Ki-67, and IL-6 in the study groups

The results indicated that the P-GP level was significantly increased in SCLC and NSCLC (10.35±0.51 and 10.06±0.32), respectively. compared with the control group (3.79±0.21). There were no significant differences between NSCLC and SCLC at p-value≤0.01. However, Ki-67 levels were significantly increased in the SCLC group (15.28±0.27), compared with both the NSCLG group (13.97±0.52) and control groups (8.43±0.24). Also, Ki-67 levels were significantly increased in the NSCLG group compared with the control group. Additionally, IL-6 was significantly increased in the SCLC (54.46±2.11 ng/mL) and NSCLC group (46.17±1.18 ng/mL) compared with the control group (26.40±1.01 ng/mL); at p-value≤0.01: Table 3.

Table 2 Indicated clinical characteristics of lung cancer patients and control

Characteristics	Patients NO. (90)	Control NO. (75)	T-test	p-value
Age (years)				
Mean±SE	65.2±0.82	65.16±0.60	3.346	0.061^{NS}
BMI				
Mean±SE	25.4±0.23	25.5±0.22	1.334	0.092^{NS}
Sex (M/F)	52/38	43/32	-	0.068^{NS}/0.0566^{NS}
No (%)	(57.7%)/(42.2%)	(57.4%)/(42.5%)	-	
Lung cancer type				
SCLC (No/%)	30/(33.3%)	-	-	0.0013**
NSCLC (No/%)	60/(66.6%)			
Stages of lung cancer				
No (%)				
I	15/(16.6%)			
II	19/(21.1%)			
III	25/(27.7%)	-	-	0.014*
IV	31/(34.4%)			

*p-value≤0.05, **p-value≤0.01, M=male, F=female, BMI=body mass index, NSCLC=non-small cell lung cancer, SCLC=small cell lung cancer NS=no significant difference, SE=standard error

Table 3 Comparison between difference groups in P-GP, Ki-67 levels

Group	P-GP (ng/ml) Mean±SE	Ki-67 (ng/ml) Mean±SE	IL-6 (ng/ml) Mean±SE
NSCLC	10.06±0.32^{a}	13.97±0.52^{a}	46.17±1.18^{b}
SCLC	10.35±0.51^{a}	15.28±0.27^{b}	54.46±2.11^{a}
Control	3.79±0.21^{b}	8.43±0.24^{c}	26.40±1.01^{c}
LSD value	0.501**	0.973**	4.088**
p-value	0.0001	0.0001	0.0001

**p-value≤0.01, LSD=least significant difference, SE=standard error
Means having different letters within the same column differed significantly.

The effect of lung cancer stages and chemotherapy in P-gp, Ki-67, and IL-6 levels in patient groups

Table 4 presents the levels of P-gp, Ki-67, and IL-6 based on lung cancer stages and chemotherapy received. The means of P-gp and Ki-67 levels in serum showed no significant difference between Stage IV (11.07±0.24 and 15.17±0.25, respectively) and Stage III (10.28±0.24 and 13.90±0.58, respectively) compared to Stages II (9.85±0.21 and 12.67±0.68, respectively) and I (9.07±0.15 and 11.37±0.40, respectively). Meanwhile, IL-6 exhibited a significant increase at Stage IV (56.24±0.61) compared to other stages, though no significant differences were found between Stages III (49.2±3.14) and II (47.52±3.67).

Regarding chemotherapy, the mean P-gp level was significantly higher in patients who received chemotherapy for 2 months (14.18±0.44) compared to those who received it for 1 month (11.85±0.26) and those who had not received chemotherapy (p-value=0.0001). The mean Ki-67 level was significantly higher in patients who had not received chemotherapy (15.57±0.6) compared to those who had (p-value=0.0001). Meanwhile, IL-6 levels were significantly elevated in patients who had received chemotherapy for 2 and 1 month (66.39±2.32 and 55.58±1.91, respectively) compared to those who had not received chemotherapy (51.4±2.67; p-value=0.027).

Determination of P-gp gene expression by using real-time PCR technology

Table 5 indicates the mRNA expression of P-GP in the study groups. The P-GP mRNA expression average had a significant increase in both the SCLC (7.07±0.10), and NSCLC groups (6.63±0.12) compared with the healthy control group (2.18±0.12): p-value<0.05. When comparing the concentration of P-GP using RT-PCR, it was observed to be higher than that detected by ELISA. The average P-GP level in patients divided by the average in the control group was three times higher in patients when analyzed through RT-PCR, compared to 2.6 times higher when assessed by ELISA.

Assessment of the diagnostic sensitivity, specificity, PPV, NPV, and accuracy of study parameters in lung cancer patients and control group

The ROC analysis of P-gp, Ki-67, and IL-6 concentrations in the lung cancer patients and control are shown in Table 6 and Figure 1. The best cut-off value for P-gp was 10 ng/ml. P-gp levels greater than 10 demonstrated a specificity of 99% and sensitivity of 100% for lung cancer diagnosis. The best cut-off value for Ki-67 was 11 ng/ml. Ki-67 levels greater than 11 demonstrated a specificity of 94% and sensitivity of 95% for lung cancer diagnosis. The best cut-off value for IL-6 was 40 ng/ml. IL-6 concentrations greater than 40 ng/ml demonstrated a sensitivity of 95% and specificity of 94% for lung cancer diagnosis.

Estimates of progression-free survival (PFS) in lung cancer patients

PFS was calculated from the lung cancer diagnosis until the first chemotherapy cycle to determine disease progression or death from any cause. Progression was defined as disease advancement, indicated by tumor growth or specific clinical signs of worsening (e.g., new lesions, increased tumor size). The follow-up period for patients was 12 months. Based on the cutoff values of the study parameters, patients were divided into high (for P-gp, Ki-67, and IL-6) or low groups. Results showed a significant difference in the PFS rate between the high P-gp level group (7 months) and the low P-gp level group (12 months; p-value=0.0001). A similar significant difference was observed between the high Ki-67 (9 months) and IL-6 (10 months) groups compared to the low groups (12 months for both parameters; p-value=0.0001). These findings

suggest that high levels of P-gp, Ki-67, and IL-6 predict poor outcomes in lung cancer patients, while lower levels correlate with better responses to chemotherapy and longer PFS, indicating their potential as prognostic biomarkers.

Table 4 Effect of Stages and chemotherapy in P-gp, Ki-67, and IL-6 level of the lung cancer patient group

Parameters	Mean ± SE		
	P-GP (ng/ml)	Ki-67 (ng/ml)	IL-6 (ng/ml)
Stages			
I	9.07±0.15^{c}	11.37±0.40^{c}	45.03±2.22^{b}
II	9.85±0.21^{bc}	12.67±0.68^{bc}	47.52±3.67^{b}
III	10.28±0.24^{ab}	13.90±0.58^{ab}	49.2±3.14^{ab}
IV	11.07±0.24^{a}	15.17±0.25^{a}	56.24±0.61^{a}
LSD value	0.827**	1.432**	6.14*
p-value	0.0001	0.0001	0.020
Chemotherapy			
No Therapy (n=73)	9.17±0.35^{c}	15.57±0.6^{a}	51.4±2.67^{c}
Receiving chemotherapy for 1 month (n=11)	11.85±0.26^{b}	12.47±0.88^{ab}	55.58±1.91^{bc}
Receiving chemotherapy for 2 month (n=6)	14.18±0.44^{a}	9.90±0.76^{bc}	66.39±2.32^{ab}
LSD value	0.917**	1.332**	4.121*
p-value	0.0001	0.0001	0.027

*significant (p-value≤0.05), **significant (p-value≤0.01), LSD=least significant difference, SE=standard error, a, b, c=the difference is significant between means at the 0.05 level

Table 5 Average of MDR1 (P-GP) gene expression in the study groups

Groups	Average of MDR1 folding	p-value
NSCLC group	6.63±1.3^{a}	0.001*
SCLC group	7.07±2.11^{a}	
Control	2.18±1.5^{b}	
Average of NSCLC/ Average of Control	3.04^{a}	0.021*
Average of SCLC/ Average of Control	3.24^{a}	
Average of Control/ Average of Control	1.00^{b}	

*significant (p-value≤0.05), a, b=The mean difference is significant at the 0.05 level, NSCLC=non small cell lung cancer, SCLC=small cell lung cancer, MDR1=multidrug resistance 1

Table 6 Sensitivity, Specificity, PPV, NPV and Accuracy for study parameters in lung cancer

Parameter	Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
P-gp	10	100.0	99.0	97.0	100.0	97.7
Ki-67	11	95.0	94.0	96.7	84.0	92.7
IL-6	40	95.0	94.0	95.2	88.2	95.0

PPV=positive predictive value, NPV=negative predictive value

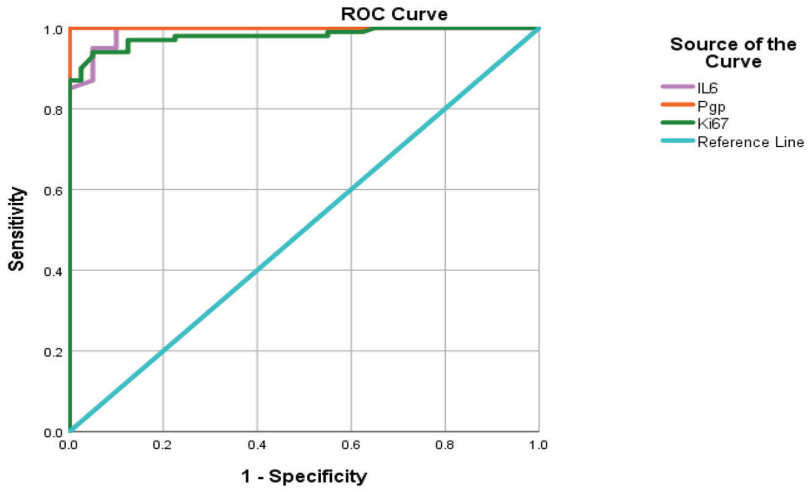

Figure 1 ROC curve analysis for P-gp levels (lung cancer patients versus controls). P-gp receiver operating characteristic (ROC) area under the curve (AUC)=1.000; 95% CI, 1.000–1.000. Ki-67 ROC AUC=0.980; 95% CI, 0.960–0.999. IL-6 ROC AUC=0.991; 95% CI, 0.979–1.000. The sensitivity and specificity of lung cancer diagnoses were more than 90%

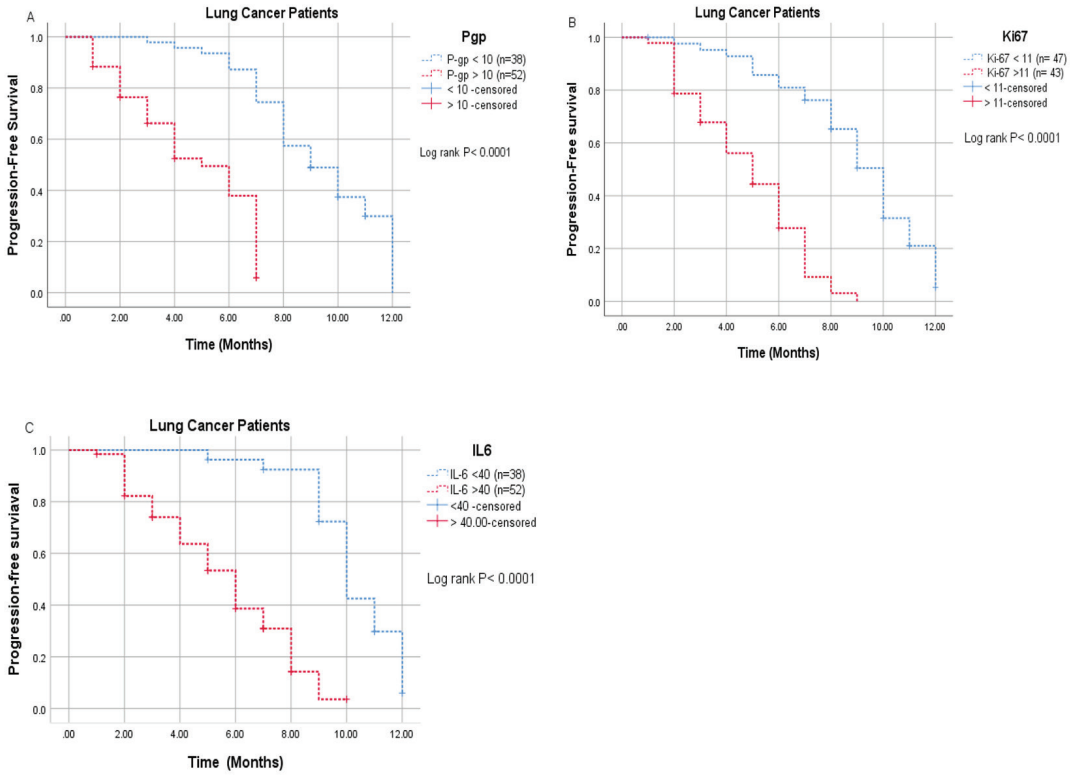

Figure 2 KaplanMeier curves for progression-free survival (PFS), according to high P-gp and a low P-gp groups (A), high Ki-67 and a low Ki-67 groups (B), and high IL-6 and a low IL-6 groups (C) in lung cancer patients

Discussion

Our study indicates the P-gp level had a significant increase in SCLC and NSCLC compared with the control group; however, there was no significant difference in levels of P-gp among NSCLC and SCLC. The expression of the P-gp, encoded by the human MDR1 gene, is one of the pathways that is known to contribute to MDR[18,19]. These results agree with the previous results by Janikova, et al.[20], who found that P-gp expression levels were elevated in both NSCLC and SCLC. Also, our results agree with van Niekerk, et al.[21], who indicated that P-gp expression was significantly elevated in metastatic cells in four of the five patients with relapsed disease (4-12 months after initiating chemotherapy). These results provide credence to the theory that acquired multidrug resistance is induced by increased P-gp protein/MDR gene expression in human lung cancer[20]. One reason for this fact is the possibility that other MDR proteins are more crucial to the chemoresistance of malignant lung cancer. Alternatively, this phenomenon is caused by some other biological variables, such as the tumor cells' resistance to apoptosis or cell death caused by chemotoxic agents[22].

The P-gp expression has Positive correlations with clinical stages (r=0.742); however, the variation of the P-gp expression in different stages was not significant[23]. A multivariate study revealed that P-glycoprotein expression can be used to predict prognosis. Eighteen patients with high P-gp levels were among the 24 patients having received postoperative adjuvant treatment and full resection. Of the 18 patients, 7 remain free of tumor recurrence, while 11 relapsed and 9 passed away due to tumor-related causes. These observations point to a bias in favor of a shorter life for P-gp cancer patients due to the possibility that P-glycoprotein is associated with chemoresistance. Therefore, detection of the expression of P-glycoprotein will aid in planning suitable adjuvant treatment for lung cancer patients, and it serves as a chemotherapeutic indicator[22]. Numerous investigations using human tumor cell lines have demonstrated a correlation between P-gp/MDRI overexpression in vitro and mechanisms of multidrug resistance[21,24].

Our results disagree with the results by Roy et al.[25], who indicated that low levels of P-gp in NSCLC and normal lung tissue. Also, these results disagree with the previous results of Berger et al.[26], who found that P-gp expression levels were similar in lung cancer and surrounding normal tissues. It is difficult to distinguish whether the observed multidrug resistance phenotype is caused by the activated production of P-gp in resistant cancer cells or by the clonal selection of inherently P-gp-positive cancer cells[20,27]. According to our results, P-gp expression measured by RT-PCR was observed to be higher than that measured by ELISA. However, used primers that were specific to the human MDR gene in our RT-PCR test, so these results might imply that there are differences in the mRNA or protein expression levels of MDR1 in lung cancer. Additionally, it is important to remember that there was no significant correlation between the mRNA and protein levels of MDR1. These results concur with those of Roy et al.[25], who indicated that genes may be transcribed, but not translated; as found by the observation that MDR1 P-gp protein was expressed by nearly 61% of NSCLC patients, but no mdr1 mRNA was identified in any of them. Different sensitivities for analytical techniques' (For example; RT-PCR for mRNA versus ELISA for protein) could be one reason for this[28]. Additionally, P-gp expression was not always consistent at the protein and mRNA levels, suggesting that the possibility of post-transcriptional regulation or the separation of protein and mRNA[29]. Consequently, greater investigation into the potential mechanisms of drug-related proteins may be helpful in reducing intrinsic resistance and creating more intrinsic lung cancer treatments in humans[7].

Ki-67 is indicative of the proliferative activity of tumor cells in lung cancer[30], and its expression varies considerably

according to the predominant histological subtypes of lung cancer[31]. Our results show a significant difference in Ki-67 levels between SCLC and NSCLC: with higher Ki-67 expression being noted in SCLC. These results agree with Folescu et al.[9], who noticed high expression of Ki-67 in SCLC, compared to NSCLC. Our results disagree with Grant et al.[32], who indicated there was no statistically significant relationship between Ki-67 and metastases of SCLC and NSCLC. These results also disagree with Ozkaya[33], who found no significant differences in the mean Ki-67 index with morphologic patterns of lung cancer. Previous studies have reported that high Ki-67 expression in lung cancer patients was associated with a poor survival outcome; these reflect biologically aggressive lung cancer and larger tumor size, confirming its prognostic validity[12]. A higher Ki-67 level indicates a higher proportion of cells in the process of division, reflecting increased tumor cell proliferation; this explains increased Ki-67 in lung cancer[30]. The results obtained by Folescu et al.[9], emphasized a linkage between Ki-67 level and the histological tumor subtype, which is in line with our study. Some studies have shown that higher Ki-67 expression indicates tumor cell proliferation, metastasis, and recurrence of cancer cells; thus, it is being used as a marker to evaluate proliferation in NSCLC and other tumors[21].

The higher the tumoral proliferation activity and proliferative potential, the lower the survival. Ki-67 can be considered as a supplementary test that helps in histological classification of tumors[31]. As Ki-67 is correlated with the prognosis, it suggests the possibility of using them as factors in assessing the proliferation status and clinical behavior[9]. High expression of Ki-67 might also be an indicator of shortened progression-free survival time[11]. In many tumors, the Ki-67 index, as an effective biomarker, has been used to predict treatment and has been used as a prognostic indicator in both breast cancer and lung cancer[10].

IL-6 has been implicated in tumor progression of lung cancer[15], and in addition, high levels of IL-6 are associated with a poor prognosis[14]. Patients with high circulating IL-6 have responded poorly to chemotherapy[34]. Therefore, a high level of circulating IL-6 has been associated with an inferior response and survival outcome in lung cancer patients treated with chemotherapy[35]. Circulating IL-6 might be secreted from immune and stromal cells in response to tumor progression and from cancer cells[16]. A high circulating IL-6 level, in turn, might facilitate tumor cell proliferation and immune invasion[35]. This study found IL-6 levels were significantly higher in the lung cancer group than those in the healthy group. Our results agree with the results by Liu et al.[36], in China, Nico la et al.[37], in Italy, and Shill et al.[38], in Bangladesh, who all found that IL-6 increased in lung cancer patients compared with their control groups. The meta-analysis revealed that IL-6 levels are higher in lung cancer patients, indicating that they could be used as a biomarker for diagnosing and prognosis lung cancer without complications[39]. Elevated levels of IL-6 in a host with a tumor are the result of increased production from T lymphocytes, predominantly from CD4 cells; which secrete Th2 cytokines[40]. IL-6 can be secreted directly by tumor cells; however,. regardless of the source, IL-6 can facilitate tumor proliferation and immune invasion[13]. Serum IL-6 levels have been identified as a prognostic factor for poor outcomes in various cancers, but little is known about their value in immunotherapy[16]. IL-6 could be used as a promising molecular biomarker to diagnose and predict the metastasis of lung cancer independent of pathological types. Hence, they could improve the specificity and sensitivity of diagnosis for lung cancer patients when they are combined with other tumor markers[15].

One limitations of this study was its sample size, as the study was limited to 90 lung cancer patients. This may not provide enough statistical power to generalize the results across different populations; especially when considering different subtypes of lung cancer. Additionally, due to the

lack of longitudinal data, this study only provides a snapshot of biomarker levels at one point in time. Longitudinal studies would be more informative in assessing how these biomarkers change with disease progression or treatment. Also, conducting the study in a single hospital may have introduced bias, limiting the generalizability of the findings to other populations.

Conclusion

The results of this study confirm that P-gp, Ki-67, and IL-6 in patients with stages (I–IV) play an important role in the diagnosis and prediction of lung cancer and are poor prognostic factors for survival. Also, this study indicates that MDR1 (the P-gp gene) is highly expressed in lung cancer patients and significantly increased with increased chemotherapy received. This highly indicates that P-gp is responsible for cancer's resistance to chemotherapy. This means that P-gp is responsible for the cancer's resistance to chemotherapy.

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Conflict of interest

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References

1. Hussain AM, Lafta RK. Cancer trends in Iraq 2000–2016. Oman Med J 2021;36:e219. doi: 10.5001/omj.2021.42.
2. Al-Khateeb ZD, Mahd LH. Prevalence of lung cancer in al najaf governorate as registered in the middle euphrates oncology center 2019-2020. J Fac Med 2022;64.
3. Dahir AK, Al-Awad AS, Al-Gazally ME. Investigation the association of the VEGFR-2 and-2578C\A polymorphism as a risk factor for incidence of lung cancer in Babylon Province. Indian J Forensic Med Toxicol 2020;14:2391.
4. Garcia-Valdecasas Gayo S, Ruiz-Alvarez MJ, Gonzalez-Gay D, Ramos-Corral R, Marquez-Lietor E, Del Amo N, et al. CYFRA 21-1 in patients with suspected cancer: evaluation of an optimal cutoff to assess the diagnostic efficacy and prognostic value. Adv Lab Med 2020;1:20200005. doi: 10.1515/almed-2020-0092.
5. Shi B, Xu FF, Xiang CP, Jia R, Yan CH, Ma SQ, et al. Effect of sodium butyrate on ABC transporters in lung cancer A549 and colorectal cancer HCT116 cells. Oncol Lett 2020;20:148. doi: 10.3892/ol.2020.12011.
6. Dallavalle S, Dobričić V, Lazzarato L, Gazzano E, Machuqueiro M, Pajeva I, et al. Improvement of conventional anti-cancer drugs as new tools against multidrug resistant tumors. Drug Resist Updat 2020;50:100682. doi: 10.1016/j.drup.2020.100682.
7. Robinson K, Tiriveedhi V. Perplexing role of P-glycoprotein in tumor microenvironment. Front Oncol 2020;10:265. doi: 10.3389/fonc.2020.00265.
8. Wang J, Wang H, Zhao L, Fan S, Yang Z, Gao F, et al. Down-regulation of P-glycoprotein is associated with resistance to cisplatin and VP-16 in human lung cancer cell lines. Anticancer Res 2010;30:3593-8.
9. Folescu R, Levai CM, Grigoraş ML, Arghirescu TS, Talpoş IC, Gîndac CM, et al. Expression and significance of Ki-67 in lung cancer. Rom J Morphol Embryol 2018;59:227-33.
10. Wang D, Chen D, Zhang C, Chai M, Guan M, Wang Z, et al. Analysis of the relationship between Ki-67 expression and chemotherapy and prognosis in advanced non-small cell lung cancer. Transl Cancer Res 2020;9:3491.
11. Wang D, Ye W, Shi Q. Prognostic value of Ki-67 expression in advanced lung squamous cell carcinoma patients treated with chemotherapy. Cancer Manag Res 2021;13:6429-36.
12. Spiliotaki M, Neophytou CM, Vogazianos P, Stylianou I, Gregoriou G, Constantinou AI, et al. Dynamic monitoring of PD-L1 and Ki67 in circulating tumor cells of metastatic non-small cell lung cancer patients treated with pembrolizumab. Mol Oncol 2023;17:792-809.
13. Chonov DC, Ignatova MMK, Ananiev JR, Gulubova MV. IL-6 activities in the tumour microenvironment. Part 1. Open Access Maced J Med Sci 2019;7:2391.

14. Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. Nat Rev Cancer 2021;21:481-99.
15. Ke W, Zhang L, Dai Y. The role of IL-6 in immunotherapy of non-small cell lung cancer (NSCLC) with immune-related adverse events (irAEs). Thorac Cancer 2020;11:835-9.
16. Park CK, Chung C, Oh IJ, Kim YC, Park D, Kim J, et al. Baseline serum interleukin-6 levels predict the response of patients with advanced non-small cell lung cancer to PD-1/PD-L1 inhibitors. Immune Netw 2020;20.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001;25:402-8.
18. Chen P, Kuang P, Wang L, Li W, Chen B, Liu Y, et al. Mechanisms of drugs-resistance in small cell lung cancer: DNA-related, RNA-related, apoptosis-related, drug accumulation and metabolism procedure. Transl Lung Cancer Res 2020;9:768. doi: 10.21037/tlcr.2020.03.10.
19. Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. Cancer Drug Resist 2019 Jun;2:141. doi: 10.20517/cdr.2019.15.
20. Janikova M, Zizkova V, Skarda J, Kharaishvili G, Radova L, Kolar Z. Prognostic significance of miR-23b in combination with P-gp, MRP and LRP/MVP expression in non-small cell lung cancer. Neoplasma 2016;63:576-87. doi: 10.4149/neo_2016_419.
21. Van Niekerk A, Wrzesinski K, Steyn D, Gouws C. A novel NCI-H69AR drug-resistant small-cell lung cancer mini-tumor model for anti-cancer treatment screening. Cells 2023;12:1980. doi: 10.3390/cells12151980.
22. Hanssen KM, Haber M, Fletcher JI. Targeting multidrug resistance-associated protein 1 (MRP1)-expressing cancers: Beyond pharmacological inhibition. Drug Resist Updat 2021;59:100795. doi: 10.1016/j.drup.2021.100795.
23. Bukowski K, Kciuk M, Kontek R. Mechanisms of multidrug resistance in cancer chemotherapy. Int J Mol Sci 2020;21:3233. doi: 10.3390/ijms21093233.
24. Nair A, Amalraj A, Jacob J, Kunnumakkara AB, Gopi S. Non-curcuminoids from turmeric and their potential in cancer therapy and anticancer drug delivery formulations. Biomolecules 2019;9:13. doi: 10.3390/biom9010013.
25. Roy S, Kenny E, Kennedy S, Larkin A, Ballot J, De Villarreal MP, et al. MDR1/P-glycoprotein and MRP-1 mRNA and protein expression in non-small cell lung cancer. Anticancer Res 2007;27:1325-30.
26. Berger W, Setinek U, Hollaus P, Zidek T, Steiner E, Elbling L, et al. Multidrug resistance markers P-glycoprotein, multidrug resistance protein 1, and lung resistance protein in non-small cell lung cancer: prognostic implications. J Cancer Res Clin Oncol 2005;131:355-63. doi: 10.1007/s00432-005-0654-4.
27. Mannoush SH, Thaker AA, Jabir MS. Inhibition of ovarian cancer cells growth using gold nanoparticles and silica coated gold nanoparticles: In-vitro study. J Pharm Negative Results 2022;13:727-33. doi: 10.4103/jpnr.JPNR_38_20.
28. Hout DR, Schweitzer BL, Lawrence K, Morris SW, Tucker T, Mazzola R, et al. Performance of a RT-PCR assay in comparison to FISH and immunohistochemistry for the detection of ALK in non-small cell lung cancer. Cancers 2017;9:99. doi: 10.3390/cancers9080099.
29. Kong J, Qiu Y, Li Y, Zhang H, Wang W. TGF β1 elevates P-gp and BCRP in hepatocellular carcinoma through HOTAIR/miR 145 axis. Biopharm Drug Dispos 2019;40:70-80. doi: 10.1002/bdd.2162.
30. Mitchell KG, Parra ER, Nelson DB, Zhang J, Wistuba II, Fujimoto J, et al. Tumor cellular proliferation is associated with enhanced immune checkpoint expression in stage I non–small cell lung cancer. Int J Clin Exp Pathol 2019;158:911-9.
31. Li Z, Li F, Pan C, He Z, Pan X, Zhu Q, et al. Tumor cell proliferation (Ki-67) expression and its prognostic significance in histological subtypes of lung adenocarcinoma. Lung Cancer 2021;154:69-75.
32. Grant L, Banerji S, Murphy L, Dawe DE, Harlos C, Myal Y, et al. Androgen receptor and Ki67 expression and survival outcomes in non-small cell lung cancer. Horm Cancer 2018;9:288-94.
33. Ozkaya N. Pulmonary pathology journal club: April 2018. Am J Respir Crit Care Med 2018;197:949-52.
34. Fisher DT, Appenheimer MM, Evans SS. The two faces of IL-6 in the tumor microenvironment. Semin Immunol 2014;26:38-47.
35. Chang CH, Hsiao CF, Yeh YM, Chang GC, Tsai YH, Chen YM, et al. Circulating interleukin 6 level is a prognostic marker for survival in advanced non-small cell lung cancer patients treated with chemotherapy. Int J Cancer 2013;132:1977-85.
36. Liu Y, Gao Y, Lin T. Expression of interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) in non-small cell lung cancer and its relationship with the occurrence and prognosis

of cancer pain. Ann Palliat Med 2021;10:12759-66.

37. Nicola S, Ridolfi I, Rolla G, Filosso P, Giobbe R, Boita M, et al. IL-17 promotes nitric oxide production in non-small-cell lung cancer. J Clin Med 2021;10:4572.
38. Shill MC, Biswas B, Kamal S, Islam M, Rima SS, Ferdausi FA, Bepari AK. Screening of plasma IL-6 and IL-17 in Bangladeshi lung cancer patients. Heliyon 2023;9.
39. Kalali D, Hadjiyianni A, Isaakidou A. The use of interleukin-6 as a biomarker of lung cancer: a systematic review and meta-analysis. J Cancer Res Ther 2023;19(Suppl 2):S485-9.
40. Chen J, Li X, Huang C, Lin Y, Dai Q. Change of serum inflammatory cytokines levels in patients with chronic obstructive pulmonary disease, pneumonia and lung cancer. Technol Cancer Res Treat 2020;19:1533033820951807.