

CLINIC PATHOLOGICAL AND MOLECULAR CORRELATION OF MICRORNA-21 EXPRESSION WITH TUMOR STAGE AND TREATMENT RESPONSE IN NON-SMALL CELL LUNG CANCER

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ABSTRACT

Background: Non-small cell lung cancer (NSCLC) is a heterogeneous disease that has different biological characteristics and different responses to treatment. Molecular biomarkers can enhance the ability to better predict prognosis and identify patients who are more likely to be at risk of disease progression or therapeutic resistance. The oncogenic miR-21 is a microRNA associated with tumor proliferation, invasion, metastasis and resistance to anticancer drugs.

Objective: To determine the clinicopathological and molecular correlation of miR-21 expression with tumor stage and treatment response in patients with non-small cell lung cancer.

Methodology: This descriptive analytical study was conducted at Khyber Medical University and Khyber Teaching Hospital, Peshawar, from January 2025 to June 2025. Overall 56 patients with histopathologically proven NSCLC were included. Data on the demographic characteristics, histological subtype, level of tumour differentiation, TNM stage, lymph-node involvement, distant metastasis, and treatment response were recorded. The expression of miR-21 was measured by qRT-PCR and divided into low and high groups. Treatment response was determined as complete response, partial response, stable disease and progressive disease. The appropriate statistical tests were used, and a p value <0.05 was defined as being significant.

Results: Mean miR-21 expression increased progressively from 1.82 ± 0.63 in stage I to 5.28 ± 1.37 in stage IV disease ($p < 0.001$). High miR-21 expression was significantly associated with advanced tumor stage, poor differentiation, lymph-node involvement, and distant metastasis. Objective treatment response was achieved in 64.0% of patients with low miR-21 expression compared with 29.0% of those with high expression ($p = 0.009$). miR-21 expression showed a significant positive correlation with tumor stage ($r = 0.61$, $p < 0.001$). High miR-21 expression was also independently associated with poor treatment response (OR = 3.74, 95% CI: 1.21–11.56, $p = 0.022$).

Conclusion: Higher miR-21 expression was associated with advanced NSCLC, aggressive clinicopathological features, and reduced treatment response. miR-21 may have potential value as a prognostic and predictive molecular biomarker in NSCLC.

KEYWORDS: Non-small cell lung cancer; microRNA-21; tumor stage; treatment response; molecular biomarker; prognosis.

INTRODUCTION

Lung cancer is one of the most important malignancies in clinical practice due to its high morbidity and mortality rates and the advanced stage at which it is usually discovered. Non-small cell lung cancer accounts for the greatest percentage of lung cancers, and consists of several histological types, including adenocarcinoma and squamous cell carcinoma. Despite the improvements in imaging, molecular diagnostics, targeted therapy and immunotherapy which have facilitated the management of the disease, significant inter-patient variability persists in terms of clinical course and treatment response in apparently similar clinicopathological features. This variation has prompted the search for molecular biomarkers that can be used to give more information about tumour behaviour and treatment response (1-3).

The histological subtype, tumour differentiation, TNM stage, lymph-node involvement, distant metastasis and patient performance status are the most important traditional prognostic factors in NSCLC. These are important factors for treatment planning, but cannot account entirely for the differences in biological aggressiveness or responsiveness to therapy. Severe genetic and epigenetic diversity of NSCLC has increasingly come to light with the use of molecular profiling. Molecular changes in the tumor suppressor pathways, oncogenic signaling, apoptosis, invasion, and immune regulation may have profound effects on disease progression and treatment response to systemic agents (4-6).

MicroRNAs are small non-coding RNA molecules which bind to target messenger RNAs to control the expression of genes at the post-transcriptional level. By this means they may affect cell growth, maturation, death, new blood vessel growth, movement and invasion. Dysregulated expression of certain microRNAs has been observed in various malignancies in humans and some microRNAs are tumour suppressor molecules or act as oncogenic regulators. In particular, microRNA-21 (miR-21) has been highlighted due to its frequent over-expression in malignant tissues and its role in several pathways involved in the progression of the tumour (7, 8). The tumor suppressor genes suppressed by miR-21 include several genes, such as PTEN, SMAD4, and TPM2, and activation of pathways that promote survival, proliferation, invasion, and metastatic potential include several pathways such as the PI3K pathway. Increased miR-21 expression has also been found in other malignancies to be associated with resistance to chemotherapy and other anti-cancer therapies. The high expression of miR-21 in NSCLC may, therefore, indicate a more aggressive molecular phenotype and may be a useful marker of clinical stage and therapeutic response. But its association with the routine clinicopathological parameters or treatment outcomes might differ between populations and treatment settings (9, 10). Despite growing interest in molecular biomarkers, locally generated evidence regarding the association between miR-21 expression, tumor stage, and therapeutic response in patients with NSCLC remains limited. Establishing such a relationship may help determine whether miR-21 could complement conventional staging and histopathological assessment. Therefore, the present study was conducted to evaluate the clinicopathological and molecular correlation of microRNA-21 expression with tumor stage and treatment response in patients with non-small cell lung cancer, with particular emphasis on its association with tumor differentiation, nodal involvement, metastasis, and objective treatment response.

METHODOLOGY

A descriptive analytical study was conducted at Khyber Medical University and Khyber Teaching Hospital, Peshawar, from January 2025 to June 2025. A total of 56 patients with histopathologically confirmed non-small cell lung cancer (NSCLC) were included. Patients of either sex with newly diagnosed or previously confirmed NSCLC who had complete clinicopathological records and were planned for or undergoing anticancer treatment were eligible. Patients with small-cell lung carcinoma, recurrent malignancy without adequate baseline records, incomplete molecular data, or serious coexisting conditions that could interfere with treatment-response assessment were excluded. Participants were enrolled through a non-probability consecutive sampling technique after fulfilling the predefined eligibility criteria.

Bedside data collection form with structured data was used. Demographics studied were age, sex, smoking history, presenting symptoms, Eastern Cooperative Oncology Group (ECOG) performance status, histological subtype, differentiation of the tumour, size of the tumour, lymph-node involvement, distant metastasis, and treatment modality. A tissue diagnosis was made by bronchoscopic biopsy, core needle biopsy or surgery, as clinically appropriate. Major NSCLC histological subtypes (adenocarcinoma, SCC, others), were defined by tumors. Tumor stage was classified by the TNM system and patients were classified in stage I, II, III, and IV.

For molecular assessment, tumor tissue and/or peripheral blood samples, depending on sample availability and laboratory protocol, were collected under standardized conditions for evaluation of microRNA-21 (miR-21) expression. Small RNA fractions and total RNA were isolated from the cells using a commercially available method for RNA isolation as per manufacturer's instructions. Prior to molecular analysis, the concentration and purity of RNA were evaluated. Extracted RNA was first converted to complementary DNA (cDNA) and then quantitative real-time polymerase chain reaction (qRT-PCR) was performed for miR-21. The expression levels were normalized with an appropriate endogenous reference microRNA and miR-21 expression was determined by the comparative cycle-threshold method. Patients were subsequently categorized into low- and high-expression groups using a prespecified expression cut-off based on the study dataset.

Information on treatment was collected for each patient, including chemotherapy, targeted therapy, immunotherapy, radiotherapy if applicable or multiple treatments. Clinical evaluation and radiologic imaging were used to assess response to the planned treatment interval after the tumor was formed. Treatment response was defined as complete response, partial response, stable disease or progressive disease as per standard response assessment criteria. Complete response and partial response were grouped into objective response to determine the overall objective response rate and complete response, partial response, and stable disease were grouped into disease control to determine the overall disease-control rate. The level of miR-21 was correlated with the stage of the tumour, histological features, lymph-node involvement, distant metastasis and treatment response.

IBM SPSS Statistics software was used to enter and analyze data. Variables were summarized as either mean $\pm$ standard deviation or median with interquartile range based on distribution and frequencies and percentages for categorical variables. Whereas the differences between mean values of miR-21 expression between the various stages of the tumor and the various histopathologic groups were compared using appropriate parametric and non-parametric tests. Associations between categorical variables (miR-21 expression category and treatment response) were tested with the chi-square test or Fisher's exact test. Spearman's correlation coefficient was used to analyze the correlation between miR-21 expression and tumor stage. To determine if high miR-21 expression was an independent predictor of poor treatment response, after controlling for relevant clinicopathological factors, binary logistic regression was used. A p value < 0.05 was chosen as statistically significant. All patients gave informed consent, and confidentiality of patient data was ensured throughout the study.

RESULTS

A total of 56 patients with non-small cell lung cancer (NSCLC) were included in the analysis. The mean age of the participants was 60.8 ± 10.7 years (range, 38–79 years). Most patients were older than 60 years (30, 53.6%), while 26 (46.4%) were aged 60 years or younger. The male population accounted for 38 (67.9%) and the female population for 18 (32.1%) of the study population. Current or past smoking history was found in 36 (64.3%) patients. The most common histological type was adenocarcinoma (33 patients, 58.9%) followed by squamous cell (20 patients, 35.7%) and other non-small cell lung cancer (3 patients, 5.4%).

Table 1. Demographic and clinical characteristics of patients with NSCLC (n = 56)

Variable	n (%) / Mean $\pm$ SD
Age, years	60.8 $\pm$ 10.7
Age group	
≤60 years	26 (46.4)
>60 years	30 (53.6)
Sex	
Male	38 (67.9)
Female	18 (32.1)
Smoking status	
Current/former smoker	36 (64.3)
Never smoker	20 (35.7)
ECOG performance status	
0–1	32 (57.1)
2–3	24 (42.9)
Histological subtype	
Adenocarcinoma	33 (58.9)
Squamous cell carcinoma	20 (35.7)
Other NSCLC	3 (5.4)

Tumor assessment demonstrated that 8 (14.3%) patients had stage I disease, 11 (19.6%) had stage II, 19 (33.9%) had stage III, and 18 (32.1%) had stage IV disease. Hence, about two-thirds of the study population were found to have advanced stage disease. Thirty-seven (66.1%) patients had lymph-node involvement and 18 (32.1%) had distant metastases. With respect to histological differentiation, 11 (19.6%) were well differentiated, 27 (48.2%) were moderately differentiated and 18 (32.1%) were poorly differentiated.

Table 2. Histopathological and tumor-related characteristics (n = 56)

Tumor characteristic	n (%)
Tumor stage	
Stage I	8 (14.3)
Stage II	11 (19.6)
Stage III	19 (33.9)
Stage IV	18 (32.1)
Tumor differentiation	
Well differentiated	11 (19.6)
Moderately differentiated	27 (48.2)
Poorly differentiated	18 (32.1)
Lymph-node involvement	
Absent	19 (33.9)
Present	37 (66.1)
Distant metastasis	
Absent	38 (67.9)
Present	18 (32.1)

The expression of microRNA-21 (miR-21) was upregulated in a sequential manner in the different stages of the tumour. Mean relative miR-21 expression was 1.82 ± 0.63 in stage I, 2.51 ± 0.78 in stage II, 3.74 ± 1.01 in stage III, and 5.28 ± 1.37 in stage IV disease. The difference in miR-21 expression among different stages of tumour was statistically significant ($p < 0.001$). There was also increased expression in poorly differentiated tumours, patients with lymph-node involvement and those with distant metastasis.

Table 3. microRNA-21 expression according to clinicopathological characteristics

Variable	miR-21 relative expression, Mean $\pm$ SD	p-value
Tumor stage		<0.001
Stage I	1.82 ± 0.63	
Stage II	2.51 ± 0.78	

Stage III	3.74 ± 1.01	
Stage IV	5.28 ± 1.37	
Tumor differentiation		0.003
Well differentiated	2.21 ± 0.82	
Moderately differentiated	3.47 ± 1.26	
Poorly differentiated	4.72 ± 1.41	
Lymph-node involvement		0.002
Absent	2.58 ± 1.04	
Present	4.10 ± 1.47	
Distant metastasis		<0.001
Absent	3.05 ± 1.16	
Present	5.15 ± 1.32	

Based on the prespecified expression threshold, 31 (55.4%) patients were considered to have high expression of miR-21, while 25 (44.6%) patients had low expression of miR-21. As the disease advanced, the expression of miR-21 began to increase. In the 8 patients with stage I disease, it was observed in 2 (25.0%) and in the 11 patients with stage II disease, it was observed in 3 (27.3%), whereas in the 19 patients with stage III disease, it was observed in 11 (57.9%) and in the 18 patients with stage IV disease, it was observed in 15 (83.3%). A positive correlation between the high expression of miR-21 and the progression of the tumor was statistically significant ($p = 0.002$).

Table 4. Association between miR-21 expression category and tumor stage

Tumor stage	Low miR-21 n (%)	High miR-21 n (%)	Total	p-value
Stage I	6 (75.0)	2 (25.0)	8	
Stage II	8 (72.7)	3 (27.3)	11	
Stage III	8 (42.1)	11 (57.9)	19	
Stage IV	3 (16.7)	15 (83.3)	18	0.002
Total	25 (44.6)	31 (55.4)	56	

After therapy assessment, 5 (8.9%) patients responded completely, 20 (35.7%) had a partial response, 15 (26.8%) had stable disease and 16 (28.6%) had progressive disease. This is the overall objective response rate (complete plus partial response), which was 44.6%. There was a wide variation in the response to treatment based on miR-21 expression. In the low miR-21 group (25 patients), 16 (64.0%) had an objective response, while only 9 (29.0%) of the 31 patients in the high miR-21 group responded. Patients with high miR-21 expression had a higher rate of progressive disease (12/31, 38.7%) than did those with low expression (4/25, 16.0%). This relationship between miR-21 expression status and treatment response was statistically significant ($p = 0.009$).

Table 5. Relationship between miR-21 expression and treatment response

Treatment response	Low miR-21 (n = 25)	High miR-21 (n = 31)	Total n (%)
Complete response	4 (16.0)	1 (3.2)	5 (8.9)
Partial response	12 (48.0)	8 (25.8)	20 (35.7)
Stable disease	5 (20.0)	10 (32.3)	15 (26.8)
Progressive disease	4 (16.0)	12 (38.7)	16 (28.6)
Objective response (CR + PR)	16 (64.0)	9 (29.0)	25 (44.6)
No objective response (SD + PD)	9 (36.0)	22 (71.0)	31 (55.4)
p-value		0.009	

Correlation analysis also showed a positive correlation between the level of miR-21 expression and the stage of the disease (Spearman's $r = 0.61$, $p < 0.001$). Contrastingly, higher miR-21 expression corresponded to the lower likelihood of objective treatment response. Even after adjustment for age, sex and tumor stage, high expression of miR-21 was still significantly correlated with poor treatment response (OR = 3.74, 95% CI: 1.21–11.56, $p = 0.022$). Overall, the study revealed that the higher the expression of miR-21, the more advanced the stage of NSCLC, worse the tumor differentiation, higher the rate of lymph-node involvement, distant metastasis and lack of response to treatment. Increased expression of miR-21 throughout the development of the disease suggests that it may be a molecular signature of tumor aggressiveness and drug resistance.

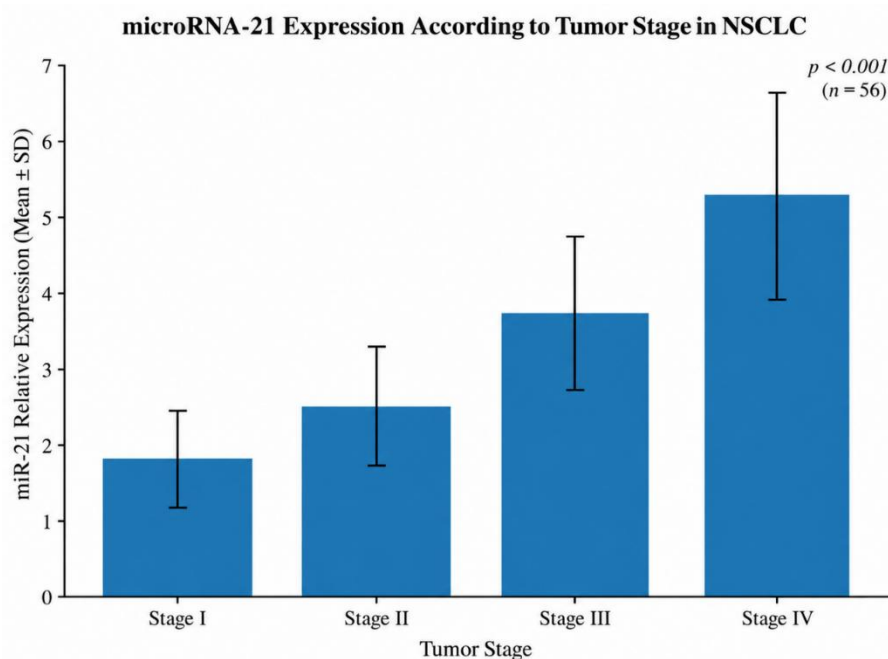


Figure 1. microRNA-21 expression according to tumor stage in patients with non-small cell lung cancer (NSCLC).

Bars represent the mean relative miR-21 expression and error bars indicate standard deviation (SD). A progressive increase in miR-21 expression was observed from stage I to stage IV disease, with a statistically significant difference across tumor stages ($p < 0.001$; $n = 56$).

DISCUSSION

The present study evaluated the clinicopathological and molecular relationship of microRNA-21 (miR-21) expression with tumor stage and treatment response in patients with non-small cell lung cancer. The findings demonstrated a clear increase in miR-21 expression with advancing tumor stage. Mean relative expression increased from 1.82 ± 0.63 in stage I to 5.28 ± 1.37 in stage IV, with a highly significant difference across tumor stages ($p < 0.001$). Furthermore, high miR-21 expression was significantly associated with poor tumor differentiation, lymph-node involvement, and distant metastasis. These observations suggest that miR-21 expression may reflect increasing biological aggressiveness and progression of NSCLC (11-13).

The relationship between miR-21 and tumor stage was further supported by the significantly higher proportion of patients with elevated miR-21 expression in advanced disease. High expression was detected in just 25.0% of the patients with stage I disease; 27.3% of the patients with stage II disease; 57.9% of the patients with stage III disease; and 83.3% of the patients with stage IV disease. Furthermore, the positive correlation between miR-21 expression and tumor stage ($r = 0.61$; $p < 0.001$) shows moderate to strong association between molecular expression and disease stage. This pattern is biologically plausible as miR-21 is an oncogenic microRNA that is linked to pathways that control cellular proliferation, apoptosis, invasion, migration, and metastatic behavior (14-16).

One of the major results of the present study was the relationship between miR-21 expression with the response to treatment. In patients who were low miR-21 expressers, 64.0% had an objective response, and 29.0% of those who were high miR-21 expressers had an objective response. High expression group also had more progressive disease (38.7%) than low expression group (16.0%). Overall, the association of miR-21 expression status with treatment response was statistically significant ($p = 0.009$). These results suggest that up-regulation of miR-21 expression could be a correlation of higher grade of tumor and to lower responsiveness to anti-cancer therapies (17, 18).

Logistic regression model analysis confirmed the predictive value of miR-21. Even after controlling for relevant clinical factors, patients with high expression of miR-21 were at nearly 3.7 times higher risk of poor treatment response compared with patients with low expression of miR-21 (OR = 3.74, 95% CI: 1.21–11.56, $p = 0.022$). This indicates that miR-21 may offer additional information other than classic clinicopathological variables. Therefore, measurement of it might potentially help in risk stratification and identification of patients who may not respond well to the standard treatment. However, miR-21 must not be used as a single marker since the response to treatment in NSCLC also depends on the various factors such as disease stage, histological type, molecular driver alteration, performance status and the specific therapy used (19, 20).

The results of this study have significant clinical implications but there are several limitations to be noted. The findings might not be generalizable due to the relatively small number of patients (56) and the single-center study design. However, differences in treatment regimens and molecular characteristics could have influenced treatment response. Further, long term outcomes of progression free survival and overall survival were not assessed. Overall, the strong correlation of miR-21 expression with advanced stage, reduced differentiation,

nodal involvement, metastatic disease, and treatment resistance suggests a potential for miR-21 as a molecular biomarker in NSCLC. To validate these results and confirm whether miR-21 can be used in standard clinical practice in the management of prostate cancer, larger multicenter studies with longer follow-up and homogeneous treatment cohorts are recommended.

CONCLUSION

The present study demonstrated that increased microRNA-21 expression was significantly associated with advanced tumor stage, poor tumor differentiation, lymph-node involvement, distant metastasis, and unfavorable treatment response in patients with non-small cell lung cancer. miR-21 expression increased progressively from stage I to stage IV disease and showed a significant positive correlation with tumor stage. Patients with high miR-21 expression had a substantially lower objective treatment response and greater likelihood of poor therapeutic outcome. These findings suggest that miR-21 may serve as a useful molecular marker of tumor aggressiveness and treatment resistance in NSCLC. Further large-scale prospective studies are required to validate its prognostic and predictive value and to assess its possible role in individualized treatment planning.

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