ORIGINAL ARTICLE

Differentiation of Malignant Pleural Effusions from Lung Squamous Cell Carcinoma and Adenocarcinoma through FTIR Spectroscopy: A Prognostic Approach

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ABSTRACT

Introduction: Malignant pleural effusion (MPE) is common in cancer patients and is often caused by neoplastic involvement of the pleural surface. This study aimed to determine the utility of Fourier transform infrared spectroscopy (FTIR) spectral analysis and anatomical pathological differentiation in MPE, squamous cell carcinoma (SCC), and lung adenocarcinoma as prognostic predictors.
Methods: This study used a cross-sectional design at Ulin General Hospital, Banjarmasin, involving advanced lung cancer patients with MPE. A non-probability sampling technique was used to recruit 30 patients. Fourier transform infrared spectroscopy was analyzed to evaluate anatomical pathology differentiation.
Results: Differences were observed in the FTIR spectral ratios A1080/A1243 and A1080/A1170 between SCC and adenocarcinoma, with p-values of 0.026 and 0.022, respectively. Significant differences were also found in the A2959/A1545 ratio between well-differentiated and poorly differentiated adenocarcinomas, with a p-value of 0.023. The receiver-operating characteristic curve (ROC) indicated good predictive value for poorly differentiated adenocarcinoma at a cut-off value of 0.944, with a sensitivity of 50% and specificity of 100%. However, no significant correlation was found between FTIR absorbance and anatomical pathology differentiation in MPE due to SCC and lung adenocarcinoma.
Conclusion: The FTIR spectral ratios A1080/A1243 and A1080/A1170 differentiate SCC from adenocarcinoma. Fourier transform infrared spectroscopy may be an adjunct to cytology, offering a more rapid and cost-effective method for differentiating MPE.

INTRODUCTION

Malignant pleural effusion (MPE) is a common complication in cancer patients caused by neoplastic involvement of the pleural surface.[1,2] Lung cancer is the leading cause, followed by breast cancer and undetected primary malignancies.[2] In a three-year observation conducted at Persahabatan National Respiratory Referral Hospital, Jakarta, 52.4% of MPE cases were found to be associated with lung cancer.[3] The United States (US) reports around 175,000 MPE cases annually, whereas Indonesia has a prevalence rate of 2.7%.[4,5] Hospitals in Jakarta and Surabaya also report high rates of MPE.[6] At Ulin General Hospital, Banjarmasin, MPE was found in 44.6% of females and 55.4% of males, with squamous cell carcinoma (SCC) accounting for 13.8% and adenocarcinoma for 72% of cases.[6]

The diagnosis of MPE can be confirmed through pleural fluid cytology, which is considered a simpler and more accessible diagnostic modality compared with other methods.[7] Lung cancer is categorized into small cell carcinoma and non-small cell carcinoma, the latter of which includes lung adenocarcinoma, SCC, and large cell carcinoma.[8,9] Treatment for non-small cell lung carcinoma generally involves chemotherapy, though

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targeted therapy with tyrosine kinase inhibitors (TKI) is an option in adenocarcinoma cases with epidermal growth factor receptor (EGFR) mutations.[10] Distinguishing between lung adenocarcinoma and SCC is crucial for guiding treatment decisions and prognosis.[11] Immunohistochemistry (IHC) can be used to differentiate between these two subtypes, but it is expensive and not widely available in hospitals across Indonesia.[12]

One third of lung cancer cases produce positive cytology results for pleural effusion, highlighting the difficulty in identifying MPE. Given the high costs of conventional examination techniques such as IHC, alternative approaches such as Fourier transform infrared spectroscopy (FTIR) are being explored for their affordability.[14] Fourier transform infrared spectroscopy analysis of pleural fluid samples has shown promise in diagnosing various pulmonary diseases, with high sensitivity and specificity, and may complement cytology for more accurate diagnosis.[14–16]

To the authors' knowledge, no study has utilized FTIR to distinguish lung adenocarcinoma from lung SCC using pleural fluid in lung cancer cases. Based on this context, this study aimed to examine the FTIR spectrum and its role in anatomical pathology differentiation in MPE due to SCC and lung adenocarcinoma as prognostic predictors.

METHODS

A cross-sectional study was conducted with 30 participants at Ulin General Hospital, Banjarmasin, from January to August 2024. The inclusion criteria were as follows: advanced-stage lung cancer patients with MPE, whose cytologic examination results showed lung adenocarcinoma or lung SCC, who were undergoing treatment at Ulin General Hospital, Banjarmasin, and who were willing to participate by signing an informed consent form.

The exclusion criteria included patients with non-primary lung cancer, those who had undergone therapy, those with a chronic infection, such as tuberculosis (TB) or/an underlying hematologic disease, and immunocompromised patients, including those with human immunodeficiency virus (HIV). This study was approved by the Ethics Committee of Ulin General Hospital, Banjarmasin (No.76/VI-Reg Riset/RSUD/24).

The data obtained were analyzed using logistic regression analysis, the Mann-Whitney U test, and the t-test, with two independent variables and one dependent variable measured on a nominal scale. Logistic regression was used to assess whether the independent variable could predict the probability of the dependent variable. The t-test result was further analyzed using the area under the curve (AUC) to determine sensitivity and specificity.

RESULTS

The features of 30 lung cancer samples are displayed in Table 1. Of the total, 56.67% were males, and 63.40% were between the ages of 45 and 60 years old. Sixty percent of the samples had completed high school. Most were employed (23.33%) or housewives (26.27%). Regarding smoking status, 40% of the samples were passive smokers. A total of 96.67% had no history of malignancy. According to anatomical pathological categorization, adenocarcinoma was present in most samples (86.6%). The majority (43.33%) had an underweight body mass index (BMI).

Table 1. Research participant characteristics

Sample Characteristics	N	(%)
Age		
<45 years old	6	(20.00)
45–65 years old	19	(63.40)
>65 years old	5	(16.60)
Sex		
Male	17	(56.67)
Female	13	(43.33)
Education		
Elementary school	3	(10.00)
Junior high school	7	(23.33)
Senior high school	18	(60.00)
Higher education	2	(6.67)
Job		
Self-employed	4	(13.33)
Employee	7	(23.33)
Farmer	3	(10)
Trader	4	(13.33)
Laborer	3	(10)
Housewife	8	(26.67)
Not working	1	(3.33)
Tribe		
Banjar	22	(73.33)
Dayak	2	(6.67)
Jawa	6	(20)
Body mass index (kg/m^2)		
Underweight (<18.5), n (%)	13	(43.33)
Normal (18.5-22.9), n (%)	12	(40)
Overweight (23-24.9), n (%)	5	(16.67)
Smoking status		
Active smoker	10	(33.33)
Passive smoker	12	(40)
Former smoker	5	(16.67)
Non smoker	3	(10)
The pathological anatomy of the effusive pleura		
Adenocarcinoma	26	(86.6%)
Squamous cell carcinoma	4	(13.3%)
History of cancer		
Yes	1	(3.33)
No	29	(96.67)
Family history of cancer		
Yes		
Lung cancer	0	(0)
Other cancer	1	(3.33)
No	29	(96.67)

Table 2. Descriptive analysis of squamous cell carcinoma and adenocarcinoma with well- and poorly differentiated types

Type of Pathological Anatomy	Differentiation		Total
	Well-Differentiated	Poorly Differentiated	
Adenocarcinoma	12 (46.15%)	14 (53.84%)	26
Squamous cell carcinoma	2 (50%)	2 (50%)	4
Total	14	16	30

Thirty samples with anatomical pathology differentiation classified as well- or poorly differentiated consisted of adenocarcinoma and SCC, as shown in Table 2. Twelve samples of adenocarcinoma (46.15%) were well-differentiated, whereas fourteen (53.84%) were poorly differentiated. Among SCC cases, two samples (50%) were well-differentiated, and two (50%) were poorly differentiated.

Table 3. Description of Fourier transform infrared spectroscopy ratio in well- and poorly differentiated adenocarcinoma and squamous cell carcinoma

	Pathological Anatomy	A2959/A1545		A1650/A1545		A1080/A1545		A1080/A1243		A1080/A1170	
	Differentiation	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adenocarcinoma	Well-differentiated	0.929	0.012	1.247	0.029	0.946	0.011	1.005	0.007	1.007	0.004
	Poorly differentiated	0.939	0.010	1.258	0.017	0.952	0.011	1.003	0.004	1.006	0.004
	Difference	**0.010**	**0.002**	**0.011**	**0.012**	**0.006**	**0.000**	**0.002**	**0.003**	**0.001**	**0.000**
Squamous cell carcinoma	Well-differentiated	0.929	0.005	1.267	0.008	0.950	0.007	1.008	0.003	1.012	0.001
	Poorly differentiated	0.938	0.002	1.264	0.006	0.955	0.003	1.005	0.000	1.008	0.000
	Difference	**0.009**	**0.003**	**0.003**	**0.002**	**0.005**	**0.004**	**0.003**	**0.003**	**0.004**	**0.001**

SD: standard deviation

Based on the FTIR ratio analysis in Table 3, the differences in intensity ratio values of various wavenumbers between adenocarcinoma and SCC, concerning well- and poorly differentiated pathological anatomy (PA) conditions, provide detailed information on the variation of chemical components in these cancer cells.

This study compared FTIR ratios in well- and poorly differentiated adenocarcinoma and SCC to determine molecular differences in cancer cells. In adenocarcinoma, a higher A2959/A1545 ratio was observed in poorly differentiated cells, indicating changes in chemical composition. A similar trend was found in the A1650/A1545 ratio, suggesting alterations in molecular structures. The A1080/A1545 ratio slightly increased in poorly differentiated cells, whereas the A1080/A1243 and A1080/A1170 ratios remained relatively stable between well- and poorly differentiated samples. In SCC, the ratios between well- and poorly differentiated cells were less pronounced than in adenocarcinoma, with minimal changes observed across most ratios.

Table 4. Ratio of Fourier transform infrared spectroscopy spectra in squamous cell carcinoma and adenocarcinoma

Ratio	Squamous Cell Carcinoma	Adenocarcinoma	p-value
A2959/A1545	0.934 ± 0.006	0.935 ± 0.012	0.877[(a)]
A1650/A1545	1.266 ± 0.006	1.253 ± 0.023	0.123[(b)]
A1080/A1545	0.952 ± 0.005	0.950 ± 0.012	0.651[(a)]
A1080/A1243	1.007 ± 0.003	1.004 ± 0.005	0.026[(b)]
A1080/A1170	1.010 ± 0.002	1.006 ± 0.004	0.022[(b)]

[(a)]Independent-samples t-test; [(b)]Mann-Whitney U test

This study compared the ratio of FTIR spectrum wavelengths in SCC and adenocarcinoma using the independent-samples t-test and the Mann-Whitney U test. Five different wavelength ratios were analyzed. The results showed no significant difference between the two cancer types for three ratios: A2959/A1545, A1080/A1545, and A1650/A1545. However, significant differences were found in the A1080/A1243 and A1080/A1170 ratios. Squamous cell carcinoma exhibited higher values in these significant ratios than adenocarcinoma. The p-values for these ratios were 0.026 and 0.022, respectively, indicating statistically significant differences.

Table 5. Mann–Whitney U test results for well- and poorly differentiated squamous cell carcinoma

Ratio	Squamous Cell Carcinoma		p-value
	Well-Differentiated	Poorly Differentiated	
A2959/A1545	0.930 ± 0.000	0.94 ± 0.000	0.121[(a)]
A1080/A1545	1.265 ± 0.005	1.265 ± 0.005	0.439[(a)]
A1650/A1545	0.945 ± 0.005	0.955 ± 0.005	0.439[(a)]
A1080/A1243	1.010 ± 0.000	1.010 ± 0.000	0.102[(a)]
A1080/A1170	1.010 ± 0.000	1.010 ± 0.000	0.102[(a)]

[(a)]Mann-Whitney U test

The Mann-Whitney U test was used to analyze the PA differentiation of MPE in well- and poorly differentiated SCC, as shown in Table 5. The A2959/A1545 ratio for well-differentiated SCC was 0.930 ± 0.000, whereas for poorly differentiated SCC, it was 0.940 ± 0.000, with a p-value of 0.121, indicating no significant difference between the two. Similarly, the A1080/A1545, A1650/A1545, A1080/A1243, and A1080/A1170 ratios also showed no statistically significant differences between well- and poorly differentiated SCC. Although slight variations in the values were observed, none of these differences were statistically significant.

Table 6. Fourier transform infrared spectroscopy spectrum ratios in well- and poorly differentiated adenocarcinoma

Ratio	Adenocarcinoma		p-value
	Well-Differentiated	Poorly Differentiated	
A2959/A1545	0.929 ± 0.0116	0.941 ± 0.0095	0.023[(a)]
A1650/A1545	1.247 ± 0.0266	1.257 ± 0.017	0.069[(b)]
A1080/A1545	0.946 ± 0.0107	0.953 ± 0.0106	0.174[(a)]
A1080/A1243	1.005 ± 0.009	1.002 ± 0.005	0.648[(b)]
A1080/A1170	1.009 ± 0.005	1.007 ± 0.005	0.331[(b)]

[(a)]Independent-samples t-test; [(b)]Mann–Whitney U test

The results of the independent-samples t-test revealed a statistically significant difference in the A2959/A1545 ratio between well-differentiated and poorly differentiated adenocarcinoma in MPE. However, no significant difference was observed in the A1080/A1545 ratio between the two groups. The Mann-Whitney U test also showed no significant differences in the A1650/A1545, A1080/A1243, and A1080/A1170 ratios.

The A2959/A1545 ratio in well- and poorly differentiated adenocarcinoma was further analyzed using the AUC, with a value of 0.738, indicating that 73% of the absorbance values for this ratio could predict poor pathological differentiation. This is considered fairly good and is supported by a significance value of <0.05 (0.04). The highest Youden index value of 0.5 was obtained at a cut-off point of 0.944, with a sensitivity of 50% and a specificity of 100%.

The chi-square values from logistic regression tests on FTIR spectra for SCC and adenocarcinoma indicated no significant differences in FTIR absorbance related to MPE. The p-values for both tests were greater than 0.05 (0.954 and 0.770, respectively), suggesting no relationship between the variables analyzed. Therefore, no statistically significant relationship between FTIR absorbance and anatomical pathology differentiation in MPE for either SCC or adenocarcinoma was found.

DISCUSSION

Malignant pleural effusion is common in cancer patients due to neoplastic involvement of the pleural surface.[1,2] Lung cancer is mainly caused by long-term exposure to carcinogens, with smoking being a substantial risk factor due to deoxyribonucleic acid (DNA) damage from cigarette smoke carcinogens.[17]

Studies on lung cancer patients showed that the majority of respondents were between the ages of 45 and 65 years old, with the disease being more common in individuals over 40 years old due to weakened immune systems.[18,19] Males are more vulnerable to MPE, possibly due to a higher susceptibility to lung cancer.[19,20] Most participants in this study had a high school education and various occupations, including housewives and employees. Potential exposure to radon gas, asbestos, and wood smoke can increase the risk of lung cancer, especially in individuals who work independently or are unemployed.[21] Passive smoking was prevalent among participants, increasing the risk of lung cancer compared with non-smokers.[22] Other contributing factors include exposure to pollution, chemicals, and genetic predisposition.[23]

Malignant pleural effusion was primarily characterized by adenocarcinoma, which was more prevalent than SCC.[24,25] Participants in this study were mainly underweight or had a normal BMI, which can influence cancer risk due to malnutrition or environmental factors.[23,26] Most participants did not report exposure to carcinogens, though some had pulmonary TB. Cancer and pleural effusion can still develop as a result of unhealthy lifestyle choices, such as poor diet and lack of exercise.[27]

The independent-samples t-test conducted on the A2959/A1545 ratio in adenocarcinoma and SCC did not show a significant difference between the two types of lung cancer. A previous study reported higher lipid/protein content in lung cancer patients than healthy individuals, but this study did not find substantial variations.[28] This could be due to SCC and adenocarcinoma sharing similar biological characteristics, which may influence the outcome.[29] External factors and sample size variability might also contribute to the lack of substantial findings. The A1080/A1545 ratio did not show a significant difference in DNA levels between SCC and adenocarcinoma. Although serum DNA concentrations are often elevated in lung cancer patients, likely due to increased cell death, this difference was not significant when comparing the two cancer types.[28,30] These findings suggest that differences in DNA content may exist, but are not significant when measured using this ratio.

The Mann-Whitney U test found no significant difference in the A1650/A1545 ratio between SCC and adenocarcinoma, suggesting similar spectral characteristics. This ratio reflects protein content changes in lung tissue. A previous study indicated a lower ratio in lung cancer patients than in healthy individuals.[28] However, this study did not find a significant difference between the cancer types, suggesting comparable NH bending and CN stretching content.

The A1080/A1243 and A1080/A1170 ratios are important indicators of structural changes in nucleic acids and have been associated with lung cancer. Patients with lung cancer exhibit higher values due to increased nucleic acid concentration, resulting from cancer cells utilizing aerobic glycolysis.[28] Although these ratios may not distinguish lung cancer patients from healthy individuals, they have shown significant differences between SCC and adenocarcinoma. Adenocarcinoma is associated with higher levels of enzymes such as thymidylate synthase and dihydrofolate reductase, which impact DNA synthesis and folate metabolism, leading to distinct nucleic acid changes.[31,32] Therefore, the A1080/A1243 and A1080/A1170 ratios may serve as useful markers in identifying nucleic acid alterations between lung cancer types.

The Mann-Whitney U test assessing PA differentiation in MPE SCC suggests that lipid/protein ratios and DNA levels do not differ significantly between well- and poorly differentiated cases. The A2959/A1545 ratio did not show a significant difference, nor did the A1080/A1545, A1650/A1545, A1080/A1243, or A1080/A1170 ratios. This suggests that cell necrosis or apoptosis may increase the DNA concentration in lung cancer patients.[28,30] These findings indicate that lipid/protein ratios and DNA levels may not be reliable indicators for differentiating between well- and poorly differentiated SCC cases.

The absorbance spectrum analysis of well- and poorly differentiated tissues showed minor differences in molecular content at specific wavenumbers, suggesting variations in protein structure, peptide bonds, and heavy molecule content between the two tissue types. Variations were observed in protein content, amide A bonds, and OH group content, indicating differences in these components between SCC tissues.[33,34] A study by Bangaoil, *et al.* (2020) on malignant and benign cancer patients revealed changes in the amide, lipid, and nucleic acid regions. Benign lung cancer patients exhibited alterations in protein configuration, with higher absorbance at specific wavelengths, indicating changes in protein secondary structure.[34] Increased protein phosphorylation in malignant samples and irregular lipid use affecting cell membrane fluidity were also reported.[34] Changes in lipid content may influence malignant cell behavior, whereas differences in nucleic acid stretching suggest cell necrosis or apoptosis.[34] Glycogen levels in malignant cells may be a differentiation marker from benign tissue.[34] These subtle chemical variations may reflect differences in protein structures, water content, or key molecular interactions, potentially influencing tissue health or differentiation.

Pathological anatomy differentiation is common in non-small cell carcinomas, particularly lung adenocarcinoma. Cytopathology remains essential for diagnosing lung cancer due to its non-invasive nature. The A2959/A1545 ratio is a key parameter in distinguishing poorly and well-differentiated adenocarcinoma, with a cut-off value of 0.944 showing high specificity and sensitivity. However, one study suggested that this cut-off might not accurately predict differentiation.[35] High sensitivity and specificity are crucial for a marker to demonstrate strong diagnostic performance, highlighting the need for further research to determine a more optimal cut-off value for predicting differentiation in adenocarcinoma.[35]

The independent t-test showed a significant difference in the A2959/A1545 ratio between well- and poorly differentiated lung adenocarcinoma, with well-differentiated samples showing a mean of 0.929±0.116 and poorly differentiated samples a mean of 0.941±0.095, with a p-value of 0.023. This suggests a lipid/protein content difference between the two adenocarcinoma types. In contrast, the A1080/A1545 ratio did not show a significant difference, with a p-value of 0.174. A previous study supports these findings, reporting a higher A2959/A1545 ratio in lung cancer patients than healthy individuals.[28] The difference in lipid and protein levels is associated with aerobic glycolysis, a metabolic pathway cancer cells use to support growth and division.[28]

The Mann-Whitney U-test results for the A1650/A1545, A1080/A1243, and A1080/A1170 ratios in well- and poorly differentiated adenocarcinomas showed no statistically significant differences between the two groups, with p-values of 0.069, 0.648, and 0.331, respectively. Overall, the independent samples t-test proved effective in detecting a significant difference in the A2959/A1545 wavelength ratio, indicating that changes in lipid/protein groups do not interfere with DNA alterations in pleural effusion adenocarcinoma with PA differentiation. The A1080/A1545 ratio difference test results also support this conclusion. In contrast, the Mann-Whitney U test showed no significant differences in other ratios tested, suggesting that specific ratios may reflect distinct spectral characteristics. Statistical analysis indicates that at least one wavelength ratio differs significantly between well- and poorly differentiated adenocarcinomas despite the generally small variations in the observed values.

The comparison of absorbance spectra between well- and poorly differentiated adenocarcinoma tissues reveals similar mainly chemical compositions, with both tissue types containing proteins, lipids, and water, resulting in distinct peaks in the spectrum. Minor

differences were noted at specific wavenumbers, suggesting subtle variations in protein structure or concentration between the two groups. The peaks at 1,645 cm^{-1} and 3,309 cm^{-1} indicate differences in peptide bond vibrations in proteins and hydrogen bonding interactions between water and protein within the tissues.[33,34] Specific wavelengths associated with protein, lipid, and nucleic acid content in cancer cells can indicate cellular structure and function changes. Disruptions in protein configuration, accelerated lipid utilization, and potential damage to DNA and ribonucleic acid (RNA) are all reflected in absorbance at these wavelengths.[34]

Although well- and poorly differentiated adenocarcinoma tissues show almost identical spectral patterns, minor differences at specific wavenumbers may influence tissue conditions without significantly changing the absorbance spectrum. This suggests that differentiation between the two tissue types may result from subtle molecular-level differences in chemical composition or structural configuration.

The t-test results revealed significant differences in lipid/protein ratios between well- and poorly differentiated SCC and adenocarcinoma in the lungs.[28] De novo lipogenesis (DNL), the new fatty acid synthesis process, is implicated in cancer, particularly in lung adenocarcinoma, where abnormal lipid metabolism is common.[36] Fatty acid synthase, a key enzyme in fatty acid synthesis, is often overexpressed in lung adenocarcinoma, leading to lipid accumulation in tumour cells.[37] Fourier transform infrared spectroscopy has been used to analyze the biochemical composition of human tissue, revealing distinct metabolic profiles between adenocarcinoma and SCC.[38] Differences in lipid metabolism at the cellular level allow for differentiation between the two carcinoma types. However, principal component analysis (PCA) did not detect significant differences in the protein region between normal lung tissue in patients with SCC and the control group.[33,39]

The origin of lung adenocarcinoma from glandular cells in the peripheral areas of the lung, often with a mucin component and located closer to the pleural cavity, contrasts with SCC, which arises from squamous epithelial cells lining the respiratory tract and lacks mucin secretion. Squamous cell carcinoma typically presents as a solid mass without a fluid component, is more aggressive in growth and invasion, and is anatomically located in the central airways.[40]

Table 3 shows that adenocarcinoma with poor differentiation had higher absorbance levels than well-differentiated cases, contrary to the expected trend. This discrepancy could be attributed to inflammatory factors or substances in the pleural fluid analyzed through FTIR, potentially affecting absorbance readings. Karpathiou, *et al*. (2022) suggested that inflammatory processes in the pleura can alter fluid composition, possibly leading to misinterpretation of absorbance values and inaccurate differentiation assessment in tumours.[41]

The logistic regression tests for SCC and adenocarcinoma yielded chi-square values of 0.003 and 0.086, with corresponding p-values of 0.954 and 0.770. As both p-values exceeded 0.05, these findings indicate no significant relationship between FTIR spectrum wavelength and anatomical pathology differentiation in MPE. There is insufficient evidence to support the hypothesis of such a connection in either carcinoma type. This suggests that FTIR spectrum wavelengths are not associated with anatomical pathology differentiation in MPE for SCC or adenocarcinoma.

A study by Kaznowska, *et al*. (2018) used FTIR spectroscopy to compare cancerous and normal lung tissues. They observed changes in absorbance values and shifts in absorption bands, indicating potential differences in chemical structure, particularly in proteins, collagens, and glycogens. The shifts in wavenumbers suggest protein conformation and composition alterations between tumor and healthy tissues.[33] Although FTIR can detect molecular changes in tissues before clinical symptoms emerge, not all identified modifications may result in significant pathological alterations or clinical manifestations.[14]

The comparison of absorbance spectra between adenocarcinoma and SCC tissues revealed similarities in chemical components, with slight differences in protein content or structure.[42] Despite these differences, both types of cancer share almost identical chemical profiles, suggesting variations in function or tissue status.[33]

CONCLUSION

In conclusion, while FTIR may not directly correlate with anatomical pathology differentiation or prognosis, it can still serve as a valuable tool for detecting molecular changes in cancer, including distinguishing between different types of cancer tissues. This study shows that specific wavelength ratios from the FTIR spectrum can differentiate between cancer types and help predict prognosis. In MPE SCC and lung adenocarcinoma, the A1080/A1243 and A1080/A1170 ratios showed significant differences, whereas others did not. Similarly, when differentiating between well- and poorly differentiated adenocarcinoma, the A2959/A1545 ratio was significant. However, this study found no significant differences in the differentiation of SCC.

This study also established a cut-off value for the A2959/A1545 ratio to predict poor differentiation in adenocarcinoma. The results suggest that FTIR spectrum examination may help predict prognosis and differentiate between cancer types. In addition, it might be used as an adjunct to cytology in differentiating SCC and adenocarcinoma, particularly in resource-limited settings where IHC is unavailable.

LIMITATIONS OF THE STUDY

Some limitations of the study include the small number of samples and non-homogeneous data distribution. In pleural fluid, the presence of low cancer cell counts mixed with inflammatory components may affect the accuracy of FTIR detection. Individual variability and co-existing lung diseases could also influence the results. Further validation studies are needed to confirm the reliability of FTIR in broader clinical settings, particularly in pleural fluid analysis, where high sensitivity and specificity are essential for accurate diagnosis.

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Conflict of Interest
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Authors' Contributions
Manuscript writing: VAM. Designed the experiment: IN, ES, IKO. Reviewed and revised the manuscript: IN, ES, IKO, HH, MI, EK, AA, IS. Analyzed and interpreted data: ES, IKO. All authors contributed and approved the final version of the manuscript.

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