EVALUATION OF *PTGER4* GENE METHYLATION IN PLASMA FOR LUNG CANCER DETECTION

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Abstract

Objectives: To evaluate the diagnostic value of *PTGER4* (*Prostaglandin E Receptor 4*) gene methylation in plasma for lung cancer detection compared to healthy controls. ***Methods:*** A cross-sectional, case-control study was conducted on 149 non-small cell lung cancer (NSCLC) patients and 100 healthy individuals. Peripheral blood samples were collected for *PTGER4* methylation analysis using real-time methylation-specific PCR (MSP). Data were analyzed using SPSS 26.0, including group comparisons, receiver operating characteristic (ROC) analysis, and logistic regression. ***Results:*** The *PTGER4* methylation positivity rate was significantly higher in lung cancer patients (18.8%) compared to healthy controls (3.0%, $p < 0.001$). *PTGER4* methylation positivity increased the risk of lung cancer by 6.3 times (OR = 6.30; 95%CI: 1.90 - 20.40). The area under the ROC curve (AUC) was 0.58, with a sensitivity of 18.8% and a specificity of 97.0%. ***Conclusion:*** *PTGER4* gene methylation is a promising biomarker for lung cancer detection. Despite its limited sensitivity, the high specificity suggests its potential as a confirmatory diagnostic tool, particularly when combined with imaging modalities such as low-dose computed tomography (LDCT).

Keywords: *PTGER4* methylation; Lung cancer; Biomarker; Non-invasive diagnosis; Methylation-specific PCR.

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INTRODUCTION

Lung cancer is one of the most prevalent cancers and the leading cause of cancer-related deaths worldwide. According to the Global Cancer Observatory (GLOBOCAN), lung cancer ranks first in terms of both new cases and cancer-related deaths reported in 2022 [1]. In Vietnam, lung cancer is also among the most common cancers, particularly in men [2]. Alarmingly, the majority of patients are diagnosed at advanced stages, leading to limited treatment efficacy and reduced survival rates [3].

Early diagnosis of lung cancer plays a crucial role in improving prognosis and increasing the chances of successful treatment. Current diagnostic methods, such as LDCT and tissue biopsy, have shown significant advancements. However, they still present limitations in terms of cost, accuracy, and broad applicability [4]. Therefore, the search for non-invasive biomarkers in peripheral blood for early detection of lung cancer has become a promising research direction and has attracted significant attention within the medical community [5].

Among various biomarkers, DNA methylation, an epigenetic modification that regulates gene expression, has emerged as a potential tool for early cancer detection [6]. DNA methylation changes, especially hypermethylation in the promoter regions of tumor suppressor genes, can lead to gene silencing and contribute to cancer development. The *PTGER4* gene, a member of the prostaglandin E2 receptor family, is involved in inflammatory processes and has been implicated in tumor progression and metastasis, including lung cancer [7]. Recent studies have demonstrated that abnormal methylation of the *PTGER4* gene (*mPTGER4*) can be detected in the plasma of lung cancer patients and holds diagnostic potential [8].

Although several international studies have highlighted the potential of *mPTGER4* for early lung cancer detection, similar research in Vietnam remains limited. Therefore, this study aims to: *Evaluate the diagnostic value of PTGER4 gene DNA methylation in the peripheral blood of lung cancer patients, compared to the healthy control group.* The findings of this study will contribute to clarifying the role of *mPTGER4* as a non-invasive biomarker, supporting early detection and diagnosis of lung cancer in clinical practice.

MATERIALS AND METHODS

1. Subjects

This study included two groups: NSCLC patients and healthy controls.

The NSCLC group consisted of patients with histopathologically confirmed primary NSCLC who had not received chemotherapy, radiotherapy, or surgery before blood sampling. The control group included individuals with no history of cancer and no pulmonary lesions on chest X-rays or LDCT, matched by age and gender to the NSCLC group.

* *Exclusion criteria:* Patients with small-cell lung cancer (SCLC), secondary lung cancer, advanced chronic diseases (COPD, tuberculosis, autoimmune disorders), withdrawal of consent, or incomplete clinical data.

All participants provided written informed consent, ensuring confidentiality and the right to withdraw at any time without affecting their medical care.

* *Sample size calculation:*

The sample size was calculated using the formula for comparing two proportions to detect a significant difference in *mPTGER4* positivity between lung cancer patients and healthy controls.

$$n = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

n: Minimum required sample size per group; Z: 1.96 (for 95% confidence level); p: Expected *mPTGER4* positivity rate (15%, based on exploratory study); d: Margin of error (6%).

The calculation yielded a minimum of 91 participants per group. To enhance statistical power, the final sample sizes were 149 NSCLC patients and 100 healthy controls.

2. Methods

* *Study design:* A cross-sectional, case-control study was conducted from April 2021 to August 2024 at the National Lung Hospital and the Institute of Biomedicine & Pharmacy, Vietnam Military Medical University.

Data collection included demographics, clinical history, and biological samples.

For each participant, 10mL of peripheral blood was collected into EDTA tubes, centrifuged within 6 hours, and plasma was stored at -80°C. DNA was extracted using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany) and bisulfite-treated with the EZ DNA Methylation-Gold™ Kit (Zymo Research, USA). *mPTGER4* methylation was analyzed via real-time methylation-specific PCR (qMSP) using primer and probe sequences from Wei et al. (2021) [8]. Results were classified as positive (methylation detected) or negative (no methylation) and used for statistical analysis.

* *Study variables:*

The study included two categories of variables: Demographic variables,

including age, gender, BMI, and smoking history; and primary variables, including *PTGER4* DNA methylation test results (positive/negative). These variables were analyzed to assess the association between *mPTGER4* and lung cancer diagnosis.

* *Statistical analysis:*

Data were analyzed using SPSS 20.0. Categorical variables were summarized as frequencies (%) and compared using the Chi-square test, while continuous variables were presented as Mean ± SD and analyzed using the T-test or Mann-Whitney U test (for non-normal distributions).

The diagnostic performance of *mPTGER4* was assessed via ROC curve analysis. Logistic regression estimated the association between *mPTGER4* positivity and lung cancer risk, expressed as ORs with 95%CIs. $p < 0.05$ was considered statistically significant.

3. Ethics

This study was approved by the Ethics Committee for Biomedical Research at Military Hospital 103, Vietnam Military Medical University, under the official decision No. 182/2021/CNChT-HĐĐĐ, dated August 10, 2021. The approval ensured that all procedures adhered to the Declaration of Helsinki and Vietnamese regulations for biomedical research. The data used in this study were collected and analyzed with the approval of the Military Hospital 103, Vietnam Military Medical University. The institution has granted permission for the use and publication of the research findings in compliance with applicable regulations. The authors declare that there are no conflicts of interest related to this study.

RESULTS

1. Characteristics of study participants

The study included 149 NSCLC patients and 100 healthy controls. Table 1 presents the demographic and clinical characteristics of the study population.

The lung cancer group had a significantly higher mean age than the healthy control group (60.9 ± 8.6 vs. 54.9 ± 10.7 years, $p < 0.001$). The proportion of males was also higher among lung cancer patients (65.8%) compared to healthy controls (46.0%, $p = 0.0026$). Regarding BMI, lung cancer patients were more likely to be underweight (7.4% vs. 3.0%) and less likely to be overweight/obese (23.5% vs. 43.0%, $p = 0.0029$). Smoking history was more prevalent among lung cancer patients (57.0%), nearly 1.8 times higher than in healthy controls (32.0%, $p < 0.001$).

Table 1. Demographic and clinical characteristics of study participants.

Characteristics	Lung cancer patients (n = 149)	Healthy controls (n = 100)	p
Age (Mean ± SD, years)	60.9 ± 8.6	54.9 ± 10.7	< 0.001
Male gender (%)	65.8	46.0	0.0026
BMI (Categories, %)	Underweight: 7.4 Normal: 69.1 Overweight: 23.5	Underweight: 3.0 Normal: 54.0 Overweight: 43.0	0.0029
Smoking history (%)	57.0	32.0	< 0.001

2. *PTGER4* methylation status: Lung cancer patients versus healthy controls

To evaluate the diagnostic potential of *mPTGER4*, the positivity rates were compared between lung cancer patients and healthy controls. The results are presented in table 2.

Table 2. *mPTGER4* results by study groups.

***mPTGER4* results**	**Lung cancer patients (n = 149)**	**Healthy controls (n = 100)**	**p**
Negative	121 (81.2%)	97 (97.0%)	< 0.001
Positive	28 (18.8%)	3 (3.0%)	

mPTGER4 positivity was significantly higher in lung cancer patients (18.8%) compared to healthy controls (3.0%, $p < 0.001$). The OR for *mPTGER4* positivity in lung cancer patients was 6.3 (95%CI: 1.9 - 20.4), indicating that lung cancer patients were over 6 times more likely to test positive for *mPTGER4* than healthy individuals.

3. Diagnostic accuracy of *mPTGER4*: ROC analysis

The diagnostic performance of *mPTGER4* was further evaluated using ROC curve analysis. The AUC, sensitivity, and specificity are presented in table 3.

Table 3. Diagnostic performance of *mPTGER4* for lung cancer detection.

Index	Lung cancer patients vs. healthy controls
AUC	0.58
Sensitivity	18.8%
Specificity	97.0%

The AUC was 0.58, indicating modest diagnostic accuracy. The sensitivity was 18.8%, suggesting that *mPTGER4* alone may not be sufficient as a standalone screening test. However, the specificity was 97.0%, meaning that a positive *mPTGER4* result strongly supports lung cancer presence.

4. Logistic regression: Independent association of *mPTGER4* with lung cancer

To evaluate the independent association between *mPTGER4* positivity and lung cancer diagnosis, a multivariate logistic regression analysis was performed, adjusting for potential confounders, including age, gender, BMI, and smoking history. The results are shown in table 4.

mPTGER4 positivity remained a strong independent predictor of lung cancer, with an OR of 6.30 (95%CI: 1.90 - 20.40, $p < 0.001$). Importantly, other factors, including age, gender, BMI, and smoking history, were not statistically significant ($p > 0.05$).

Table 4. Logistic regression: The association between *mPTGER4* and lung cancer diagnosis.

Variable	Coefficient (Coef.)	OR (95%CI)	p
mPTGER4 positive	2.159	6.30 (1.90 - 20.40)	< 0.001
Age	0.027	1.03 (0.99 - 1.07)	0.208
Male gender	-0.331	0.72 (0.30 - 1.36)	0.458
BMI	-0.518	0.60 (0.30 - 1.22)	0.145
Smoking history	-0.018	0.98 (0.95 - 1.01)	0.268

DISCUSSION

These findings highlight the potential of *mPTGER4* as a non-invasive biomarker for lung cancer detection. The significantly higher positivity rate in lung cancer patients compared to healthy controls aligns with previous studies, including the study by Schotten et al. (2021) reported elevated *mPTGER4* in lung cancer patients compared to those with benign pulmonary nodules and chronic obstructive pulmonary disease [3]. The high specificity (97.0%) observed in this study suggests that *mPTGER4* is useful for confirming lung cancer diagnosis, particularly when imaging findings are inconclusive. However, the modest sensitivity (18.8%)

indicates that *mPTGER4* alone may not be sufficient as a primary screening tool. Instead, it should be considered as part of a multi-marker panel or used alongside imaging modalities like LDCT.

A study by Weiss et al. (2017) further supports this approach, showing that combining *mPTGER4* with other biomarkers such as SHOX2 significantly improved diagnostic accuracy for differentiating malignant from non-malignant lung disease. This approach may enhance early detection while minimizing false-positive results.

Moreover, the significant association between *mPTGER4* positivity and lung cancer (OR = 6.3, $p < 0.001$) reinforces its role as an independent biomarker. This association remained significant even after adjusting for age, gender, BMI, and smoking history, suggesting that *mPTGER4* reflects cancer-specific epigenetic changes rather than general risk factors.

Despite these promising results, the study has certain limitations. The relatively small sample size and cross-sectional design limit the ability to assess longitudinal changes and survival outcomes. Future studies with larger, multi-center cohorts and longitudinal follow-up are needed to validate these findings and explore the utility of *mPTGER4* for monitoring disease progression and treatment response.

CONCLUSION

In conclusion, *mPTGER4* is a promising biomarker for lung cancer detection, particularly for confirming diagnosis in patients with suspicious imaging findings. While its limited sensitivity precludes its use as a standalone screening tool, its high specificity makes it a valuable addition to current diagnostic approaches.

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