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A Thin Film Micro-Extraction Based Salivary Metabolomics and Chemometric Strategy for Rapid Lung Cancer Diagnosis

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INTRODUCTION: Lung cancer (LC) remains one of the leading causes of cancer-related mortality worldwide, largely due to the lack of reliable biomarkers for early detection.¹ Despite advances in diagnostic imaging and targeted therapies, the five-year survival rate remains low because most cases are diagnosed at advanced stages. Consequently, the development of sensitive, non-invasive, and cost-effective diagnostic approaches is a major clinical priority. Metabolomics, the comprehensive profiling of small-molecule metabolites, has emerged as a powerful tool for uncovering cancer-associated metabolic alterations, providing insights into tumor biology and facilitating the discovery of novel biomarkers for accurate diagnosis and disease monitoring. Among biological matrices, saliva is a promising diagnostic biofluid because it can be collected non-invasively, is simple to obtain, and reflects systemic and local metabolic changes. Recent studies have demonstrated its potential for detecting various cancers, including lung cancer, highlighting its value for biomarker-based early diagnosis.^{2,3} In this study, a novel thin-film microextraction (TFME) technique integrated with liquid chromatography-tandem mass spectrometry (LC-MS/MS) is introduced for the rapid, selective, and reproducible extraction of salivary metabolites. The developed TFME approach offers high throughput, reduced solvent consumption, and enhanced analytical performance, enabling the identification and quantification of key metabolic biomarkers associated with lung cancer. The objective of this workflow is to advance saliva-based metabolomics toward clinical translation, offering a promising avenue for the early and non-invasive diagnosis of lung cancer.

MATERIAL AND METHODS

Synthesis of SiO₂ Nanoparticles and TFME blade Preparation: SiO₂ nanoparticles were synthesized using the Stöber method, followed by post-coating with tetraethyl orthosilicate, centrifugation, washing with ethanol, and drying. The nanoparticles were incorporated into a polyacrylonitrile (PAN) matrix and coated onto steel TFME blades via a controlled dip-coating process to ensure uniform film thickness.

Participants and Sample Collection: Saliva samples were collected from 40 histopathologically confirmed lung

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cancer patients and 38 healthy volunteers following an overnight fast and an oral rinse. Ethical approval and informed consent were obtained (Ege University Ethics Committee, protocol: 15-11.1/46). Saliva samples were centrifuged, diluted (1:2), and stored at -80 °C until analysis.

TFME Sampling and Analysis: A 96-well plate system equipped with PAN/SiO₂-coated TFME blades was used for metabolite extraction (Figure 1). Blades were immersed in diluted saliva samples and rotated at 850 rpm for 150 minutes to allow analyte adsorption, followed by desorption of analytes in 0.1% formic acid for 30 minutes. Desorbed solutions were spiked with 0.5 µg/mL ornidazole as an internal standard prior to LC-MS/MS analysis.

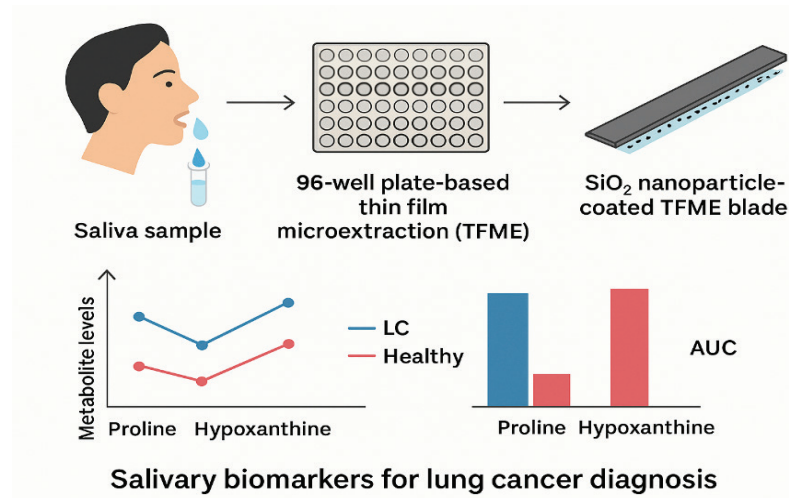


Figure 1. Graphical abstract

RESULTS: The TFME method was optimized to detect 18 metabolites in pre-treatment saliva samples from lung cancer patients. Chromatographic evaluation demonstrated that the Inertsil 100 column, employing isocratic elution with ornidazole as the internal standard, provided optimal separation efficiency and reproducibility. Extraction parameters, including desorption solution type and pH, were optimized; desorption solution type 2 at pH 8-9 yielding the highest metabolite recovery. Analytical validation indicated robust linearity (R^2 : 0.9841-0.9975), sensitivity (limit of detection: 0.014-0.97 µg/mL; limit of quantification: 0.046-3.20 µg/mL), precision (%relative standard deviation <20%), and accuracy (85-125% for most metabolites). Pathway analysis revealed significant alterations in the metabolism of phenylalanine, purine, tyrosine, histidine, and methionine. The Heatmap visualization showed increased levels of proline, hypoxanthine, phenylalanine, and tyrosine in lung cancer patients. receiver operating characteristic curve analysis highlighted these metabolites as potential biomarkers, with proline exhibiting the highest diagnostic performance [area under the curve (AUC): 0.946], followed by hypoxanthine (AUC: 0.933) and phenylalanine (AUC: 0.905).

CONCLUSION: The findings of this study demonstrate that the TFME approach is a reliable and efficient platform for metabolomic profiling in lung cancer. Using pre-treatment saliva samples, the method achieved a sensitivity exceeding 90% for detecting newly diagnosed histopathologically confirmed patients. Among the metabolites analyzed, proline, hypoxanthine, and phenylalanine showed strong diagnostic potential, consistent with the pathway analyses implicating purine and phenylalanine metabolism. These results underscore the potential of salivary metabolomics as a non-invasive screening alternative in the absence of validated early lung cancer biomarkers. Additionally, TFME's high-throughput capacity, cost-effectiveness, and environmental sustainability

support its feasibility for routine clinical application.

KEYWORDS: Biomarker, metabolomics, thin-film microextraction, LC-MS/MS, saliva

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