Multidisciplinary Cancer Investigation **Original Article**

2025, Volume 9, e6

Free fatty acids fingerprinting profile of serum from different molecular subtypes of non-metastatic breast cancer using GC-FID: a pilot study

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Submitted: 2025 August 03

Revised: 2025 September 18

Accepted: 2025 October 07

e-Published: 2025 October 22

Keywords:
Breast cancer molecular subtypes
Free fatty acids (FFA)
Serum metabolomics;
Gas chromatography–flame ionization detection (GC-FID)
Fatty acid fingerprinting;
Non-metastatic breast cancer
Metabolic biomarkers
Targeted metabolomic profiling

Background: Recent studies indicate that unusual metabolism of fatty acids is strongly associated with breast cancer (BC), offering promising evidence for finding potential BC biomarkers. This research aims to characterize serum free fatty acid (FFA) metabolic profiles and to explore possible diagnostic biomarkers for breast cancer.

Methods: In this pilot study, 12 samples were analyzed, three per breast cancer molecular subtype. Using gas chromatography with a flame ionization detector (GC-FID), we analyzed FFA fingerprint profiles across different molecular subtypes of BC. Based on existing literature, we focused on four key FFAs: linoleic acid (C18:2), stearic acid (C18:0), palmitic acid (C16:0), and oleic acid (C18:1c).

Results: Our results suggest that serum FFA fingerprinting has strong potential to distinguish between breast cancer molecular subtypes, supporting its promise as a non-invasive diagnostic approach.

Conclusion: In this study, we show for the first time that potential application of free fatty acids fingerprinting profile of serum from breast cancer molecular subtypes using GC-FID.

INTRODUCTION

Breast cancer is a widespread form of cancer in women and remains a leading cause of illness and death worldwide (1). It's a highly varied disease, with different molecular subtypes that lead to diverse clinical behaviors and responses to treatment (2). While early-stage breast cancer now has an encouraging 5-year survival rate of nearly 80% when treated promptly, outcomes for patients with advanced disease are still far from ideal because of the high risk of recurrence and metastasis (3). This makes early detection and ongoing monitoring especially critical for improving survival. Currently, the gold standard for confirming a BC diagnosis is a tissue biopsy. However, it isn't practical for routine examination. As a result, there is an urgent need for simpler, more accessible methods for diagnosing and tracking breast cancer progression (4, 5).

One of the hallmarks of cancer is metabolism (6). Metabolomics, an emerging field within systems biology, focuses on how metabolic profiles change across physiological and pathological conditions, helping uncover the underlying mechanisms of abnormal metabolism. It has become an important tool in cancer research, providing valuable insights into disease processes and helping identify potential biomarkers. Recent progress in this area suggests that metabolomic analysis in breast cancer could open new possibilities for improving patient outcomes (7).

Gas chromatography is a commonly used metabolomic tool (8). It's well recognized that accurately identifying metabolites is essential for meaningful metabolomic analysis. However, capturing a complete metabolic profile is challenging because metabolites vary widely in type and concentration. As a result, researchers are increasingly turning to targeted metabolomics, which focuses on specific groups of metabolites, thereby improving the reliability and reproducibility of findings (9).

Fatty acids, the fundamental building blocks of lipids, play essential roles in human metabolism (10). Growing evidence shows that disruptions and long-term alterations in FFA levels are thoroughly linked to cancer (11). Numerous studies have reported that fatty acid synthase–mediated lipogenesis and elevated lipolysis are key metabolic characteristics of BC. One recent study also found that elevated FFAs are associated with greater proliferation and aggression in estrogen receptor–positive BC cells (12). Overall, these findings suggest that FFAs represent a promising metabolic pool for identifying potential breast cancer biomarkers. However, most current research focuses on broad, untargeted metabolite profiling, with relatively little attention to fingerprinting of FFA patterns in breast cancer. Accurate characterization of FFA profiles—and understanding how these changes relate to breast cancer—is necessary for discovering biomarkers that may be useful in clinical practice. Blood remains the most commonly used biofluid in metabolomics research, particularly for biomarker discovery, because it is available, minimally invasive, and suitable for frequent specimen and regular screening.

In this research, we focused on FFA fingerprinting profiling of non-metastatic breast cancer with different molecular subtypes from the serum of overnight-fasted patients using GC-FID. Our outcomes confirm a strong association between FFA alterations and non-metastatic breast cancer across different molecular subtypes, offering new insights that may aid in the development of serum biomarkers for breast cancer.

MATERIALS AND METHODS

Study population

This study included 12 patients with non-metastatic breast cancer. The four non-metastatic molecular subtypes were included: Lumina A (LuA), Luminal B (LuB), Her2+ enriched (Her2+), and triple negative (TN) (Table 1). Patients were enrolled from the admittance department of the Motamed Breast Clinical Center in Tehran, Iran. Patients with BC pathologies were hospitalized for surgical management. Subsequently, histologically verified patients were included in the study. Inclusion conditions: patient aged 25–70 years; absence of any disease at the time of the study. Exclusion conditions: absence of histological confirmation of the diagnosis. This research was permitted by the Ethics Committee of the Avicenna Research Institute, ACECR.

Table 1. Features of the patients involved in the study.

Feature	Non-metastatic breast cancer, n=12
Clinical stage	
Stage IA+ IB	0
Stage IIA+ IIB	0
Stage IIIA + IIIB	11
Stage IIIC + IV	1
Lymph node status	
N_0	0
N_{1-3}	12
Subtype	
Luminal A-like	3
Luminal B-like	3
HER2-enriched	3
Triple-negative	3
HER2 status	
HER2-negative	10
HER2-positive	2
Estrogen (ER) status	
ER-negative	6
ER-positive	6
Progesterone (PR) status	
PR-negative	12
PR-positive	0
Degree of differentiation (G)	
G I + II	11
G III	1
Ki-67	
<20%	3
>20%	9

Sample collection

Blood samples were collected during the diagnosis of breast cancer molecular subtypes. Samples were collected after overnight fasting. Serum was collected after 15-30 min of blood clotting by centrifugation at 1000×g for 10 min. The supernatant was transferred to new Eppendorf tubes. Serum was stored at −80°C until analysis.

Analysis of Free Fatty Acids (FFA) in Serum

The FFA from serum samples were prepared as described before (13). Briefly, 50µL of serum was thawed for GC-FID analysis. FFA was obtained using the Folch method with chloroform/methanol (2:1, v/v). 150 µL extraction buffer was added to 50 µL of serum. The solution was mixed and centrifuged for 15 min at 4000 rpm. The supernatant, which contains FFA, was allowed to dry. To derivatize the FFA, the dried FFA was first mixed with potassium hydroxide (KOH), then with boron trifluoride (BF3) in methanol. For FFA analysis, 1 µL derivatized FFA was injected into an Agilent 7890A Plus Gas Chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a G4513A automatic liquid sampler and a flame-ionization detector. The injected FFA was separated on a 100-m capillary column (Agilent, CP-Sil 88 GC Columns, 100 m, 0.25 mm inner diameter, 0.20 µm thickness).

RESULTS

This study included four molecular subtypes of breast cancer, including LuA, LuB, Her2+, and TN. The structure of the BC molecular subtypes is shown in Table 1.

The FFA fingerprint profiles of different breast cancer molecular subtypes, as determined by GC-FID, are shown in Figure 1. As shown in the figures, a distinctive profile is achieved. Furthermore, different retention times indicate potential FFA in serum.

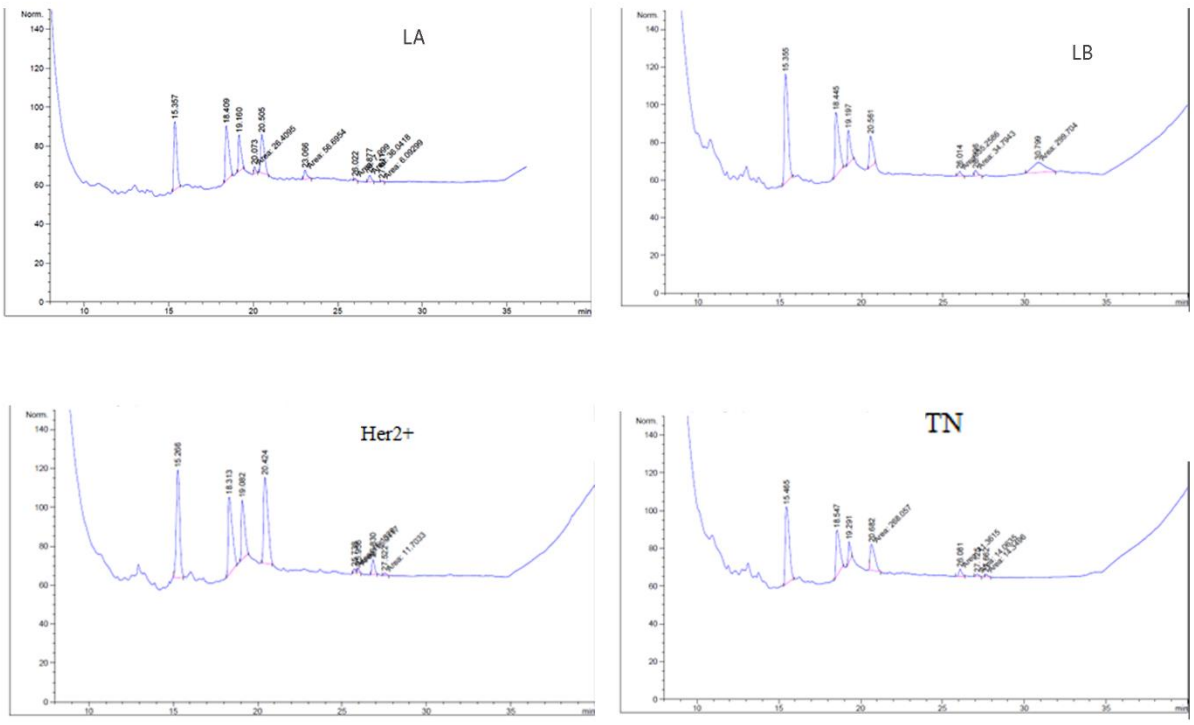

Figure 1. GC-FID FFA profile of breast cancer molecular subtypes: Luminal A (LA), Luminal B (LB), HER2+-enriched (HER2+), and Triple negative (TN). The peak intensity indicates the ratio of potential FFA.

DISCUSSION

To the best of our knowledge, our study is the first to identify the FFA fingerprinting profile of different molecular subtypes of non-metastatic breast cancer in serum, as an easy, inexpensive method for breast cancer detection. This is in agreement with the literature (14, 15). The first attempt by Lv et al. showed that FFA alterations in serum can be a good indicator of changes in breast cancer patients (14). Later, Tan et al. showed that abnormal FFA metabolism in breast cancer patients can be detected using serum FFA (15). However, this study focused on breast cancer patients rather than healthy individuals. In our study, we showed, for the first time, the FFA fingerprinting profile of distinct non-metastatic breast cancer molecular subtypes. As shown in Figure 1, six to eight distinct peaks are detected, which can be used to distinguish the non-metastatic breast cancer molecular subtypes. Based on the literature, the most intense peaks could be potentially assigned as Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1c), Linoleic acid (C18:2) (13). The exact role of these potential FFA needs to be studied. However, it is suggested by Tan *et al.* that FFA plays a role in inflammation in breast cancer (15). However, these peaks need to be confirmed by gas chromatography-mass spectrometry (GC-MS) using an internal standard. This is our limitation in this study.

CONCLUSION

We showed that GC-FID fingerprinting can be used to detect non-metastatic breast cancer molecular subtypes. Additionally, we identified potential FFa in serum based on the literature. These FFA showed that inflammation is active in non-metastatic breast cancer in different molecular subtypes. However, a limitation of our study was that we did not have an internal standard to identify potential biomarkers in FFA from serum by GC-FID. Furthermore, a more complete spectrum of FFA in serum could be obtained using GC-MS.

ACKNOWLEDGMENT

This study was supported by an INSF grant no.

98004915.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

Not applicable.

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