An Overview on Molecular Markers in Uterine Aspirates for Non-Invasive Diagnosis For Endometrial Carcinoma Detection

Rithikha Sooriyaprakash[1a], Thirumalai Kumaran Rathinam*[1b], Ramanarayana Reddy V[2], Yokesh Shanmugam[1c], Divya Chokkalingam[1d]

Affiliation:
*[1a]Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences, Chennai-602105.
*[1b]Professor and Head, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences1,Chennai-602105.
*[1c]Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences, Chennai-602105.
*[1d]Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences, Chennai-602105.
[2]Research Scholar, Saveetha College of Pharmacy, Saveetha Institute of Medical and Technical Sciences1, Chennai-602105.
*Corresponding Author's E-mail: thirumalaikumaranr.socp@saveetha.com

KEYWORDS

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Abstract:

Introduction: This research aims to enhance the diagnostic accuracy of endometrial cancer (EC), a common and debilitating malignancy prevalent among women in developed nations. The rising incidence of EC, attributed to increasing obesity and longevity, necessitates improved diagnostic methods. Traditional invasive procedures like dilation and curettage (D&C) or hysteroscopy, although effective, can cause patient discomfort and variability in diagnosis due to subjective histopathological evaluations.

Objectives: To address these limitations, this study explores non-invasive molecular diagnostics, specifically identifying biomarkers in uterine aspirates.

Methods: The investigation, conducted at Vall d'Hebron University Hospital, involved collecting uterine aspirates from patients across various EC stages and performing RNA extraction and gene expression analysis to identify potential molecular markers.

Results: The study revealed several genes with altered expression in EC tissues, including up regulated genes like ACAA1, AP1M2, CGN, and DDR1, and down regulated genes like DCN and EFEMP2. These findings were validated through techniques like RT-qPCR, immunohistochemistry (IHC), and Western blot analysis, ensuring the reliability of these markers at both RNA and protein levels.

Conclusions: Statistical analysis revealed that certain biomarkers, such as SIRT6 and P4HB, demonstrated high diagnostic accuracy with areas under the curve (AUC) approaching 1, indicating their potential to significantly enhance early EC detection, reducing the need for invasive procedures and potentially improving patient outcomes. The study concludes by emphasizing the need for further large-scale validation and clinical integration of these biomarkers, which could revolutionize EC diagnosis and treatment.

1. Introduction

Endometrial cancer (EC) poses a significant threat to women's health globally, particularly in developed nations, where it represents the most prevalent malignancy of the female reproductive system. The escalating incidence of EC, fuelled by the rising tide of obesity and increasing life

expectancy, has rendered it a critical area of focus for oncologists, researchers, and healthcare policymakers. In Europe, the annual incidence of EC ranges from 13 to 24 per 100,000 women, with a disturbing trend of increasing mortality rates in specific demographics, underscoring the urgent need for effective screening modalities that can detect EC at its nascent stage, where the disease is most amenable to treatment [1]. Current diagnostic protocols for EC primarily rely on histopathological evaluation of endometrial tissue obtained through invasive procedures like dilation and curettage (D&C) or hysteroscopy, considered the gold standard, yet fraught with limitations. The invasiveness of these procedures can lead to patient discomfort, complications, and even deter some individuals from undergoing screening, while the subjectivity inherent in histopathological interpretation can result in variability in diagnoses, leading to either over-treatment or under-treatment of the condition. In response to these limitations, the field of molecular diagnostics has gained significant traction, with molecular markers, particularly those detectable in uterine aspirates, offering a promising alternative to traditional methods. By identifying and validating these markers, the study aims to revolutionize the early detection and diagnosis of EC, thereby improving patient outcomes, reducing the burden on healthcare systems, and ultimately, saving lives [2].

2. Objective

This study explores non-invasive molecular diagnostics, specifically identifying biomarkers in uterine.

3. Methods

SAMPLE COLLECTION AND PATIENT SELECTION:

The investigative study enlisted a heterogeneous cohort of patients undergoing surgical intervention for endometrial cancer (EC) at Vall d'Hebron University Hospital, encompassing a comprehensive spectrum of disease stages, ranging from early-stage to advanced EC, thereby facilitating a thorough and nuanced understanding of the disease's complex molecular topology. The inclusion criteria ensured a diverse representation of patients, providing a rich and varied dataset for analysis. Control samples were meticulously collected from non-cancerous regions of the endometrium in the same patients, serving as a vital baseline for comparative analysis and enabling the identification of subtle molecular differences [3]. Uterine aspirates were skilfully obtained utilizing the Cornier pipelle, a minimally invasive, user-friendly device specifically designed to collect endometrial tissue without the necessity for anaesthesia, prioritizing patient comfort, ease of use, and minimizing procedural complications. This method was deliberately chosen for its potential to be widely adopted in routine clinical settings, offering a promising alternative to more invasive and burdensome procedures. To maintain the integrity and fidelity of the samples, RNA was promptly preserved post-collection, a crucial step ensuring the accuracy, reliability, and validity of subsequent molecular analyses and downstream applications [4].

GENE EXPRESSION ANALYSIS:

The study utilized a robust, multi-step approach to identify potential molecular markers, commencing with the extraction of high-quality RNA from samples using standardized protocols, ensuring contaminant removal and RNA integrity preservation. Microarray analysis, a high-

throughput screening method, was employed to examine thousands of genes simultaneously, identifying differentially expressed genes between cancerous and non-cancerous tissues. The data were analysed using the Tethys algorithm, a sophisticated bioinformatics tool, leveraging the ENSEMBL database for accurate genomic annotations. Real-time quantitative PCR (RT-qPCR) validated gene expression changes, providing a nuanced understanding of molecular alterations in EC, revealing novel biomarkers, therapeutic targets, and shedding light on the complex molecular mechanisms underlying this disease [5].

PROTEIN-LEVEL VALIDATION:

To augment the RNA-level analysis, the study also conducted a comprehensive examination of the expression of selected markers at the protein level, utilizing immunohistochemistry (IHC) to precisely localize the expression of these markers within the cellular context of the endometrium. IHC is a highly valuable technique in elucidating the distribution and intensity of protein expression, offering critical insights into the functional roles of these markers in the pathogenesis of EC [6]. Furthermore, Western blot analysis was employed to provide additional validation of the presence of these proteins in the tissue samples, allowing for the detection of specific proteins based on their molecular weight and providing further confirmation of the markers identified through RNA analysis. The synergistic combination of IHC and Western blotting ensures that the study's findings are not solely reliant on RNA expression, but are also corroborated by the actual protein products, which are the functional molecules within the cell, thereby providing a more comprehensive understanding of the molecular mechanisms underlying EC [7].

STATISTICAL ANALYSIS:

The study's statistical robustness was meticulously ensured through the application of various analytical techniques, including Receiver Operating Characteristic (ROC) curve analysis, to rigorously assess the diagnostic performance of the identified biomarkers. The area under the curve (AUC) was meticulously calculated for each marker, providing a quantitative measure of its ability to accurately distinguish between cancerous and non-cancerous samples, with an AUC approaching 1 indicative of a highly accurate biomarker and an AUC approaching 0.5 suggestive of a marker with negligible diagnostic utility [8]. Furthermore, the study employed a range of additional statistical measures, including sensitivity, specificity, and positive predictive value (PPV), to comprehensively evaluate the clinical relevance and applicability of the biomarkers in a real-world clinical setting, where the consequences of false positives or negatives can have significant implications for patient outcomes. These metrics are essential in determining the biomarkers' clinical utility, reliability, and potential for integration into routine clinical practice [9].

UNVEILINGMOLECULAR BIOMARKERS:

The comprehensive microarray analysis revealed a subset of genes exhibiting significantly altered expression profiles in endometrial cancer (EC) tissues compared to non-cancerous controls. Notably, genes such as ACAA1, AP1M2, CGN, and DDR1 demonstrated pronounced upregulation, implying their potential contribution to EC pathogenesis. These genes are intricately involved in various cellular processes, including fatty acid metabolism, vesicle-mediated transport, and cell adhesion, which are frequently dysregulated in cancerous tissues. Conversely, genes such as DCN and

EFEMP2 exhibited downregulation in EC tissues, suggesting their potential role in the disruption of extracellular matrix organization and cellular differentiation, processes that are often aberrant in cancerous tissues. The differential expression of these genes suggests their potential utility as biomarkers for EC, either individually or as part of a multi-gene panel, providing a promising avenue for the development of diagnostic and therapeutic strategies [10].

VALIDATION OF BIOMARKERS IN UTERINE ASPIRATES:

The study further corroborated the utility of these biomarkers by validating their expression in uterine aspirates, thereby confirming that the gene expression alterations observed in tissue samples were also detectable in these minimally invasive specimens. This finding holds significant promise, as it supports the feasibility of utilizing uterine aspirates for EC diagnosis, potentially mitigating the need for more invasive and burdensome procedures. The validation process entailed rigorous RT-qPCR analysis of the selected biomarkers in uterine aspirates, which consistently demonstrated differential expression patterns analogous to those observed in tissue samples. This consistency across sample types substantially reinforces the case for these biomarkers as dependable indicators of EC, underscoring their potential to revolutionize EC diagnosis and treatment [11] table 1.

TABLE 1: Summary of Key Aspects and Biomarkers Identified in Endometrial Carcinoma Detection Study

Aspect	**Endometrial Carcinoma Detection Study**
Cancer Type	Endometrial carcinoma (EC)
Prevalence	Most prevalent invasive gynaecological cancer in Western countries, with rising incidence
Importance of Early Detection	Crucial for enhancing patient outcomes
Limitations of Current Diagnostics	Current methods like biopsies are invasive, uncomfortable, and prone to variability
Study Focus	Investigation of molecular markers in uterine aspirates as a non-invasive approach to improve EC detection
Key Biomarkers Identified	**ACAA1**: Associated with fatty acid metabolism and overexpressed in EC tissues **AP1M2:** Involved in vesicle-mediated transport and overexpressed in EC tissues **CGN:** Linked to cellular junctions and overexpressed in EC tissues **DDR1:** Receptor tyrosine kinase involved in cell adhesion, significantly overexpressed in EC tissues
Validation Techniques	Real-time quantitative PCR (qPCR), Immunohistochemistry (IHC), Western blotting
Results	High sensitivity and specificity in distinguishing between cancerous and non-cancerous tissues
Potential Impact	Could revolutionize EC screening by providing a more patient-friendly and accurate diagnostic tool, enabling earlier

	intervention and improved survival rates
Future Directions	Further research needed to validate findings across diverse populations and to develop standardized protocols for clinical use, paving the way for a new era in EC diagnosis and treatment.

4. Results

PROTEIN-LEVEL VALIDATION AND FUNCTIONAL IMPLICATIONS:

The immunohistochemistry (IHC) and Western blot analyses provided additional corroborative evidence of the significance of these biomarkers, further substantiating their relevance in EC. IHC revealed that the upregulated markers were predominantly localized in the tumour epithelium, exhibiting strong staining intensity, whereas the downregulated markers displayed diminished staining, consistent with their reduced expression levels in cancerous tissues. Western blot analysis confirmed the presence of these proteins at their expected molecular weights, aligning with the gene expression data and providing further validation of the findings [12]. Furthermore, the functional roles of these proteins were also explored, with some markers being implicated in crucial cancer-related pathways, such as the PI3K/AKT and MAPK pathways, which are known to drive tumorigenesis and progression in various cancers, including EC. These findings suggest that these biomarkers may play critical roles in the pathogenesis of EC, highlighting their potential as therapeutic targets and diagnostic indicators [13].

STATISTICAL VALIDATION AND DIAGNOSTIC ACCURACY:

The Receiver Operating Characteristic (ROC) curve analysis of the biomarkers yielded exceptionally high Area Under the Curve (AUC) values, particularly for markers such as SIRT6 and P4HB, which demonstrated AUCs of 0.95 and 0.91, respectively. These values signify outstanding diagnostic accuracy, implying that these biomarkers possess the potential to serve as dependable tools for discerning EC from non-cancerous conditions with a high degree of precision [14]. Furthermore, the sensitivity and specificity of the biomarkers were remarkably high, with certain markers achieving sensitivity rates exceeding 90% and specificity rates approaching 85%. These results hold considerable promise for the development of a screening test that could be employed in a routine clinical setting, offering a minimally invasive alternative to current diagnostic methods, which often involve more invasive and burdensome procedures. The exceptional diagnostic performance of these biomarkers suggests their potential to revolutionize EC diagnosis, enabling earlier detection and treatment, and ultimately improving patient outcomes [15].

IMPLICATIONS OF MOLECULAR BIOMARKERS IN EC DIAGNOSIS:

The discovery of reliable molecular biomarkers revolutionizes EC diagnosis, offering a game-changing approach to detecting this disease. Uterine aspirates provide a minimally invasive, risk-free, and potentially earlier detection method, enabling timely interventions and improved outcomes. Biomarkers like ACAA1 and DDR1 are involved in critical cellular processes dysregulated in cancer, including metabolic reprogramming and cell adhesion. These biomarkers hold both diagnostic and therapeutic potential, while downregulated markers like DCN and EFEMP2 highlight disrupted cellular functions in EC, including extracellular matrix remodelling and cellular

differentiation. Elucidating these molecular changes can inform targeted therapies that restore normal cellular functions and improve patient outcomes [16].

COMPARISON WITH TRADITIONAL DIAGNOSTIC METHODS:

In contrast to conventional diagnostic approaches for EC, which frequently involve invasive procedures like biopsies or imaging techniques like trans-vaginal ultrasound, the utilization of molecular biomarkers offers several distinct advantages. The non-invasive nature of uterine aspirates renders them a more appealing option for patients, potentially leading to increased participation rates in screening initiatives [17]. Furthermore, molecular diagnostics can provide more objective and quantifiable results compared to the subjective interpretation of imaging or histopathology, thereby reducing diagnostic variability and ensuring that patients receive timely and appropriate treatment based on accurate diagnoses. The high sensitivity and specificity of the identified biomarkers also suggest that they could minimize the rate of false positives and negatives, which are significant issues in current diagnostic practices. False positives can lead to unnecessary treatments and patient anxiety, while false negatives can delay crucial interventions, adversely affecting patient outcomes [18].

CHALLENGES AND FUTURE DIRECTIONS:

Despite the promising findings of this study, several challenges persist in translating these biomarkers into clinical practice. One of the primary challenges is the need for large-scale validation studies involving diverse populations to confirm the generalizability of the biomarkers. Different populations may exhibit variations in gene expression profiles, which could impact the performance of the biomarkers. Additionally, the integration of molecular diagnostics into routine clinical workflows necessitates the development of standardized protocols and the training of healthcare professionals in the use of these novel tools [19]. Regulatory approval processes for new diagnostic tests can also be protracted and complex, requiring robust evidence of the biomarkers' clinical utility and cost-effectiveness. Future research should focus on addressing these challenges, including conducting multi- center studies to validate the biomarkers in broader populations and exploring their potential for guiding personalized treatment strategies. For instance, biomarkers associated with specific molecular pathways could be used to stratify patients and tailor treatments based on their individual molecular profiles, leading to more effective and targeted therapies [20].

5. Conclusion

The ground-breaking study marks a significant milestone in the pursuit of non-invasive, precise, and early detection methods for endometrial cancer. The identification and validation of molecular biomarkers in uterine aspirates offer a promising alternative to traditional diagnostic approaches, holding the potential for earlier diagnosis and improved patient outcomes. The biomarkers identified in this study, including ACAA1, AP1M2, CGN, DDR1, DCN, and EFEMP2, demonstrate strong potential for clinical application. Their high diagnostic accuracy, combined with the non-invasive nature of uterine aspirate collection, makes them ideal candidates for routine screening initiatives. Moving forward, the successful translation of these biomarkers into clinical practice will necessitate further validation in larger, more diverse populations, as well as the development of standardized protocols for their use. The integration of molecular diagnostics into existing healthcare systems

could significantly enhance the early detection of endometrial cancer, leading to improved patient outcomes and potentially reducing the overall burden of this disease. The future of endometrial cancer diagnosis lies in the continued exploration and validation of molecular markers, and this study provides a solid foundation for these efforts. By advancing our understanding of the molecular changes associated with endometrial cancer, we can move closer to a future where early detection is not only possible but routine, leading to more effective treatments and, ultimately, better survival rates for patients worldwide.

Data Availability Statement:

This article contains no datasets generated or analyzed during the current study.

Conflicts of interest

The author declares no conflicts of interest.

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