Circulating miRNA 27a and miRNA150-5p; a noninvasive approach to endometrial carcinoma

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Abstract
The search for novel non-invasive biomarkers such as epigenetic molecular markers is new hope for common and burdensome cancers. We aim to assess serum expression of miRNA 27a and miRNA150-5p in endometrial cancer patients. Serum was drawn for 36 un-intervened endometrial cancer patients scheduled for hysterectomy and 35 controls. miRNA 27a and miRNA150-5p were measured by real time reverse transcription polymerase chain reaction. Significant overexpression of both miRNA in patients (p < 0.001). At cutoffs 0.2872 & > 1.02, miRNA 27a showed 100% sensitivity, specificity, positive and negative predictive values. miRNA150-5p showed 88.89% sensitivity, 100% specificity, 100% positive and 78.9% negative predictive values. Areas under curve were 1.0 for miRNA 27a, 0.982 for miRNA 150 performing much better than Ca125. miRNA 27a was significantly associated with type I endometroid endometrial cancer. Conclusion: miRNA 27a and miRNA-150-5P can be suggested as promising biomarkers of endometrial cancer possibly part of a miRNA panel for management.

Keywords  Epigenetics · Endometrial carcinoma · miRNA 27a · miRNA15-5p · Ca125

Introduction
Endometrial cancer (EC) incidence has doubled in the last 20 years with the increasing burden of obesity and is also expected to increase in developing countries. Incidence has increased steadily due to changes in lifestyle of women, delayed marriage and lower gravidity, which make it one of the major lethal cancer of all gynecologic malignancies. It is the commonest female genital malignancy worldwide, and its prevailing histological type is endometrial endometrioid adenocarcinoma [1, 2].

EC diagnosis depends on the combination of ultrasound, magnetic resonance imaging, and serological markers but none of them are sufficient. Ca125 is as a serum marker for EC diagnosis and screening but is now recognized to have poor specificity as other gynecologic malignancies such as ovarian cancer can also show the raise of Ca125. Thus, the search for and validated biomarkers for EC is needed to improve diagnosis [3].

Epigenetic molecular mechanisms include RNA-based machinery. Regulatory noncoding RNAs including microRNAs (miRNAs) constitute the most important class in most tissues. MiRNAs are located intracellularly but have also been detected in the circulation and in body fluids [4]. MiRNA synthesis pathway is well documented [5–9]. MiRNAs encompass nuclear functions as the regulation of gene expression at transcriptional level [10].

MiRNAs play a role in the human endometrium development and in endometriosis [11]. Uterine miRNAs changes in its expression. This has been proposed to play a possible role in endometrial pathologies [12]. Several factors have been established for endometrial cancer including, factors for metastasis, oncogenic pattern and invasion MiRNAs differ in expression pattern from that of normal endometrium

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Several miRNAs have been identified as involved in oncogenesis, invasion and metastasis [14–16]. Other are under expressed in endometrial cancer including mir-let7e and mir-R-30c [14, 15, 17].

MiRNA 27a is a member of miR-23a–27a–24–2 cluster. Levels of the three family members vary in expression in different pathological conditions. They are highly expressed in acute lymphoblastic leukemia, and acute myeloid leukemia, but they are down-regulated in acute promyelocytic leukemia [18]. MiRNA 27a has been confirmed as a regulator in important pathological processes including osteoarthritis [19], viral infections [20], adipocyte differentiation [21] cell proliferation and fat metabolism [22], and multidrug resistance [23]. Its oncogenic role has been illustrated by its upregulation in osteosarcoma [24], colon carcinoma [25] and breast cancer [26]. This role was demonstrated in tumor promoting functions as increasing cancer severity and chemotherapeutic agents resistance [27].

MiRNA150-5p serve as a biomarker of human lymphocyte activation. In various types of hematopoietic malignancies, it has been reported to be downregulated, including myelodysplastic syndrome, leukemia and lymphoma [28]. MiRNA150-5p aberrant expression has been associated with cancer development by affecting tumor suppressor genes and oncogenes [29]. In certain solid tumors, the functions and regulatory mechanism of miRNA150-5p as an oncogene or tumor suppressor gene are variable. High expression levels have been identified in gastric and breast cancer while its expression was found to be decreased in oesophageal squamous cell carcinoma [30–32]. MiRNA-150 overexpression downregulated the expression of the pro-apoptotic gene, early growth response factor 2 in gastric cancer [33]. While in breast cancer, blocking miRNA-150 action with inhibitors in cell lines resulted in cell death and ectopic expression of miRNA-150 promoted growth and clonogenicity, and reduced apoptosis [30].

The aim of this work was to evaluate the serum expression level of miRNA 27a and miRNA150-5p in endometrial cancer patients and to correlate their levels with different clinical and laboratory findings as well as tumor staging.

### Materials and methods

#### Subjects

Thirty-six un-intervened patients who were diagnosed with endometrial cancer attending Shatby Alexandria University hospital in the period between July 2018 till February 2019 and scheduled for surgery were included in this study. Also, 36 age and sex matched healthy subjects were included as a control group. Patients with other malignancies, systemic diseases (as hepatic; renal; cardiac or respiratory diseases), sepsis, and collagenic diseases (as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis) were excluded. Serum expression level of miRNA 27a and miRNA150-5p were measured by real time polymerase chain reaction RT-PCR.

#### Gene expression study

Total serum RNA was isolated using miRNeasy Mini Kit (QIAGEN) Cat No./ID: 217004, according to the manufacturer’s protocol. Each sample, as a spike-in control received 5 μl of 5 nM Syn-cel-miR-39 (miScriptmiRNA Mimic) and then total RNA was purified from 400 μl of sample. Phenol/ guanidine-based lysis was used in the miRNeasy Mini Kit. It uses silica membrane-based purification of total RNA. Qiazol Lysis Reagent was used to homogenize samples. Addition of chloroform and centrifugation separated the homogenate into aqueous and organic phases. RNA was extracted in the upper aqueous phase and ethanol was added to provide appropriate binding conditions total RNA. The sample was then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane and solutions used to wash away phenol and other contaminants efficiently. RNase-free water elutes high quality RNA. The concentration of total RNA was quantified by a Nanodrop 2000 (Nanodrop, USA). The range of results was 11–73. ng/μl. TaqMan MicroRNA Reverse Transcription (RT) Kit, Applied Biosystem was used for the reverse transcription reaction. The recommended reaction volume was 20 μL. The plate was prepared and ABI prism 7900 sequence detection system (Ambion, USA) was used for amplification and detection by RT-PCR. Differences in serum miRNA 27a and miRNA150-5p expression were normalized to cel-miR-39, determined with the Livak ΔCt method, and reported as $2^{-\Delta \Delta Ct}$ (relative expression). It was calculated as follows:

$\Delta Ct$: is the difference of CT value between the target gene and spike in reference genes (cel-miR-39).

#### Relative quantification miRNA expression in patients

$\Delta Ct = Ct$ (target miRNA) − Ct (reference miRNA)

$\Delta \Delta Ct$: is the difference of $\Delta Ct$ value between the cases and the average of control ($\Delta \Delta Ct = \Delta Ct$ cases − average $\Delta Ct$ control).

The relative quantification of miRNA expression was presented as the fold change normalized to the spike in reference mirna 39 and relative to the healthy control samples.

#### Relative quantification miRNA expression in healthy control

$\Delta Ct = \Delta Ct$ of control − average $\Delta Ct$ of control
**Statistical analysis**

Quantitative data of the present work was analyzed, using F-test (ANOVA) and post hoc test (Scheffe) for pair wise comparison. All statistical calculations were performed using IBM SPSS software package version 20.0, where p < 0.05 was considered statistically significant.

All study participants signed informed consents. Faculty of Medicine Research Ethics Committee in University of Alexandria approved this study and was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

**Results**

Characteristics of the study sample are illustrated in Table 1. Relative expression of miRNA 27a and 150-5p were both significantly overexpressed in endometrial cancer patients than control serum samples. This suggests the potential role for both micro RNAs in differentiating endometrial cancer from healthy females. (p < 0.001) (Fig. 1).

For diagnosis of endometrial cancer, the overall accuracy of miRNA 27a relative expression at a cutoff of 0.2872 was surprisingly 100%. miRNA 150-5p relative expression had an overall accuracy of 98.2% at a cutoff 1.02 while the traditional Ca 125 serum marker had an overall accuracy of 82.2% at a cutoff 12.23 U/mL. At the above mentioned cutoffs, miRNA 27a showed 100% sensitivity and specificity, positive and negative predictive values of 100%. miRNA150-5p showed 88.89% sensitivity and 100% specificity, 100% positive and 78.9% negative predictive values. Areas under the curve were 1.0 for miRNA 27a, 0.982 for miRNA150-5p which were higher than serum Ca 125 which was a mere 0.822.

Table 2 and Fig. 2 show the accuracy measures (at cutoff point with the highest sensitivity and specificity) of the conventional serum parameter Ca 125 and miRNA 27a and miRNA150-5p as proposed new markers.

A significant association between miRNA 27a and tumour type was found; as it was overexpressed in endometrial cancer type I than type II. miRNA150-5P was overexpressed in postmenopausal when compared to premenopausal endometrial cancer patients. No other associations could be detected.

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**Table 1** Comparison between the two studied groups according to different parameters

| Characteristics                         | Endometrial cancer patients (n = 36) | Controls (n = 36) | Test of sig. | p    |
|----------------------------------------|--------------------------------------|-------------------|--------------|------|
| Age (years)                            | Mean ± SD 57.6 ± 8.3                  | 53.7 ± 9.4        | t = 1.488    | 0.143|
|                                        | Median (Min.–Max.) 59.5 (40–70)      | 54 (39–67)        |              |      |
| Ca125 (U/mL)                           | Mean ± SD 25.5 ± 15.8                 | 11.3 ± 4.5        | U = 96.0*    | <0.001*|
|                                        | Median (Min.–Max.) 19.1 (3.4–56.3)   | 9.9 (5.6–19.2)    |              |      |
| miRNA 2–ΔΔCt (relative expression)     | miRNA 27-a                            |                   |              |      |
|                                        | Mean ± SD 1.1 ± 0.5                    | 0.1 ± 0.1         | U = 0.0*     | <0.001*|
|                                        | Median (Min.–Max.) 0.9 (0.5–2.4)      | 0.1 (0–0.3)       |              |      |
| miRNA 150-5p                           | Mean ± SD 1.7 ± 0.7                    | 0.6 ± 0.3         | U = 9.50*    | <0.001*|
|                                        | Median (Min.–Max.) 1.5 (0.9–3.4)      | 0.6 (0.1–1)       |              |      |

U Mann Whitney test, t Student t-test
p p value for comparing between the two studied groups
*Statistically significant at p ≤ 0.05
as regards tumor grade, stage, depth of myometrial invasion, lymphovascular space or lymph node involvement. Table 3 describes the tumor related variables of the studied endometrial cancer patients. The association of these characteristics with miRNA 27a was described in Table 4 and with miRNA-150-5p in Table 5.

Table 2 Accuracy measures with cut-off points of serum miRNA 27a and miRNA-150-5p relative expression in prediction of endometrial cancer as opposed to Ca 125 as an old marker

|                      | AUC  | p   | 95% C.I. | Cut off | Sensitivity | Specificity | PPV | NPV  |
|----------------------|------|-----|----------|---------|-------------|-------------|-----|------|
| Ca125 (U/mL)         | 0.822 | < 0.001* | 0.701 | 0.943 | 12.23 | 83.33 | 73.33 | 88.2 | 64.7 |
| 2^-ΔΔCt (Relative expression) |      |     |          |         |             |             |     |      |
| miRNA 27a            | 1.000  | < 0.001* | 1.000 | 1.000 | 0.2872 | 100.0 | 100.0 | 100.0 | 100.0 |
| miRNA-150-5P         | 0.982  | < 0.001* | 0.955 | 1.009 | 1.02  | 88.89 | 100.0 | 100.0 | 78.9  |

AUC area under a curve, P value probability value, CI confidence intervals, LL lower limit, UL upper limit, PPV positive predictive value, NPV negative predictive value

*Statistically significant at p ≤ 0.05

Table 3 Tumor related variables in the studied cases (n = 36)

|                      | No. (%) |
|----------------------|---------|
| Menopause            |         |
| Pre                  | 9 (25%) |
| Post                 | 27 (75%)|
| Tumour Type          |         |
| I                    | 28 (77.8%)|
| II                   | 8 (22.2%)|
| Grade                |         |
| I                    | 9 (25%) |
| II                   | 22 (61.1%)|
| III                  | 5 (13.9%)|
| Stage (n = 34)       |         |
| I                    | 20 (55.6%)|
| Ia                   | 13 (38.2%)|
| Ib                   | 7 (20.6%) |
| II                   | 10 (29.4%)|
| IIIc                 | 4 (11.8%) |
| Depth of myometrial invasion (n = 34) |         |
| <50%                 | 17 (50%) |
| >50%                 | 17 (50%) |
| Lymph vascular invasion (n = 34) |         |
| Space                |         |
| Negative             | 23 (67.6%)|
| Positive             | 11 (32.4%)|
| Nodes                |         |
| Not done             | 1 (2.9%) |
| Negative             | 27 (79.4%)|
| Positive             | 6 (17.6%) |

Discussion

Endometrial cancer (EC) is a common malignant gynecological tumor where current biomarkers are not sufficient for early and accurate diagnosis. Serum miRNAs, considering their stability, may prove an effective and minimally invasive diagnostic method. Circulating miRNAs have been reported to have diagnostic significance in cases of EC. Jia W. and his colleagues were the first to study using a genome wide serum miRNA expression profiling analysis and found a four-miRNA signature, including miR-222, -23, -186, and -204 serving as a non-invasive approach for EC diagnosis [34].

In the current study, relative expression of miRNA 27a and miRNA150-5p were both significantly overexpressed in serum of endometrial cancer patients than serum of control. This suggests the potential role for both micro RNAs
in distinguishing between endometrial cancer and healthy females (p < 0.001).

Upregulation of miRNA 27a in endometrial cancer patients is in accordance with previous studies. Mozos et al., observed upregulation of miRNA 27a expression in invasive endometrioid adenocarcinoma tissue [35]. Our findings further complete this work as we confirm the upregulation of miRNA 27a in the serum of these patients which proposes it as a strong non-invasive biomarker. Also, we note that miRNA 27a was significantly associated with the same type of endometrial cancer (type I endometrioid) [35]. MiRNA 27a along with miR-15b and -22 might regulate the development of EC having diagnostic role by a specific pathway [36].

On the other hand, in cervical cancer, decrease in expression of miRNA 27a was documented. Host miRNAs were specifically regulated by HPV16 and HPV18 viruses in organotypic raft cultures vaginal keratinocytes. Also viral oncoprotein E6, E7 caused miRNA 27a under expression [37]. In a trial to understand the role of miRNA 27a as an oncogenic miRNA, it was demonstrated that an estrogen receptor when activated, can inhibit the expression of proapoptotic protein BAX through upregulating miRNA 27a. Thereby increased BCL2/BAX ratio may promote survival and proliferation causing precancerous lesions and type I endometrial adenocarcinoma [38]. Moreover the expression of FOXO1 (apoptosis factor) was targeted by miRNA 27a resulting in tumor apoptosis inhibition and cells survival [35].

The function of miRNA 27a as a tumor promoter through targeting MAP2K4 as in osteosarcoma can explain the current study finding [24], AGGF1 in bladder carcinoma [39], prohibitin in gastric carcinoma [40], and other genes that control protein transcription factors at G2-M checkpoint demonstrated in breast cancer cells [41]. Genistein anticancer agent studied in colon cancer targeted miRNA 27a also [42].

Serum miRNA150-5p was also significantly upregulated in patients in comparison to controls. This was in accordance with previous miRNA-based TCGA-UCEC project [43].

### Table 4  Association of miRNA 27a 2–ΔΔCt relative gene expression with tumor related variables (n = 36)

|                      | No. | miRNA 27a         | Test of sig. | p     |
|----------------------|-----|-------------------|--------------|-------|
|                      |     | Min.–Max. Mean ± SD Median |       |       |
| Menopause            |     |                   |              |       |
| Pre                  | 9   | 0.5–1.4 1 ± 0.3 0.9 | U = 113.00  | 0.774 |
| Post                 | 27  | 0.5–2.4 1.1 ± 0.6 0.8 |              |       |
| Tumor Type           |     |                   |              |       |
| I                    | 28  | 0.5–2.4 1.2 ± 0.6 0.9 | U = 54.0*   | 0.027*|
| II                   | 8   | 0.6–1.3 0.8 ± 0.3 0.7 |              |       |
| Grade                |     |                   |              |       |
| I                    | 9   | 0.5–1.1 0.8 ± 0.2 0.7 | H = 5.583   | 0.061 |
| II                   | 22  | 0.6–2.4 1.2 ± 0.6 0.9 |              |       |
| III                  | 5   | 0.6–1.3 1.0 ± 0.4 0.9 |              |       |
| Stage (n = 34)       |     |                   |              |       |
| Ia                   | 13  | 0.5–2.4 1.2 ± 0.6 1.1 | H = 4.469   | 0.215 |
| Ib                   | 7   | 0.6–2.2 1.4 ± 0.7 1.7 |              |       |
| II                   | 10  | 0.6–0.9 0.8 ± 0.1 0.8 |              |       |
| IIIc                 | 4   | 0.7–1.3 1.0 ± 0.4 1.0 |              |       |
| Depth of myometrial invasion |     |                   |              |       |
| <50%                 | 17  | 0.5–2.4 1.3 ± 0.6 1.1 | U = 94.0    | 0.085 |
| >50%                 | 17  | 0.6–1.7 0.9 ± 0.4 0.8 |              |       |
| Lymph vascular space |     |                   |              |       |
| Negative             | 23  | 0.5–2.4 1.1 ± 0.6 0.8 | U = 119.0   | 0.800 |
| Positive             | 11  | 0.6–1.7 1.1 ± 0.5 0.9 |              |       |
| Lymph nodes          |     |                   |              |       |
| Negative             | 27  | 0.5–2.4 1.1 ± 0.6 0.8 |              |       |
| Positive             | 6   | 0.7–1.3 1.0 ± 0.3 0.9 |              |       |

*Statistically significant at p ≤ 0.05
which focused on miRNA sequences downloaded from The Cancer Genome Atlas Project. MiRNA150-5p was differentially expressed as demonstrated by the difference of miRNA profile between metastatic and nonmetastatic ECs using bioinformatics technique. It was demonstrated to regulate multiple pathways of cancer, including the Wnt, NOTCH, and TGF-β signaling by functional enrichment analysis.

In cancer cervix also miRNA150-5p played an important role. In invasive cervical squamous cell carcinomas 68 upregulated miRNAs were identified including miRNA150-5p [44]. Li et al. [45] demonstrated that the level of miRNA150-5p expression was higher in the advanced stage of cervical cancer and in cervical intraepithelial neoplasia which is a well-defined precursor stages of squamous cell carcinomas [46]. In serum samples from cervical cancer patients expression of miRNA150-5p patients was also increased. MiRNA150-5p promoted the proliferation, migration and invasion of human cervical cancer cells HeLa and SiHa cells. [47].

| Table 5 Association of miRNA-150-5P 2−ΔΔCt relative gene expression with tumor related variables (n = 36) |
|---------------------------------------------------------------|
| **No.** | **miRNA-150-5P** | **Test of sig.** | **p** |
|---------------------------------------------------------------|
| **Min.–Max.** | **Mean ± SD** | **Median** | **U = 47.0* | **0.005** |
| **Menopause** | | | | |
| Pre | 9 | 0.9–3 | 1.4 ± 0.9 | 1 | U = 47.0* | 0.005* |
| Post | 27 | 1.1–3.4 | 1.7 ± 0.7 | 1.5 | | |
| **Tumor Type** | | | | |
| I | 28 | 0.9–3.2 | 1.6 ± 0.7 | 1.4 | U = 82.0 | 0.267 |
| II | 8 | 1.3–3.4 | 1.9 ± 0.9 | 1.5 | | |
| **Grade** | | | | |
| I | 9 | 1–1.7 | 1.3 ± 0.3 | 1.1 | H = 5.179 | 0.075 |
| II | 22 | 0.9–3.4 | 1.9 ± 0.8 | 1.5 | | |
| III | 5 | 1.1–1.4 | 1.3 ± 0.2 | 1.4 | | |
| **Stage (n = 34)** | | | | |
| Ia | 13 | 1.0–3.0 | 1.6 ± 0.7 | 1.5 | H = 5.536 | 0.137 |
| Ib | 7 | 1.1–3.4 | 2.4 ± 1.0 | 3.0 | | |
| II | 10 | 0.9–1.7 | 1.3 ± 0.3 | 1.4 | | |
| IIIc | 4 | 1.4–1.6 | 1.5 ± 0.1 | 1.5 | | |
| **Depth of myometrial invasion** | | | | |
| <50% | 17 | 1.0–3.2 | 1.7 ± 0.8 | 1.5 | U = 137.0 | 0.812 |
| >50% | 17 | 0.9–3.4 | 1.6 ± 0.7 | 1.4 | | |
| **Lymph vascular space** | | | | |
| Negative | 23 | 1.0–3.4 | 1.8 ± 0.8 | 1.5 | U = 103.0 | 0.403 |
| Positive | 11 | 0.9–1.8 | 1.4 ± 0.3 | 1.4 | | |
| **Lymph nodes** | | | | |
| Not done | 1 | 1.1 | | | U = 52.0 | 0.189 |
| Negative | 27 | 1.0–3.4 | 1.8 ± 0.8 | 1.5 | | |
| Positive | 6 | 0.9–1.6 | 1.3 ± 0.3 | 1.4 | | |

U Mann Whitney test, H Kruskal Wallis test, p p value for comparing between the different categories

*Statistically significant at p ≤ 0.05

#Excluded from the comparison due to small number of case (n = 1)

Targets involved in the cell proliferation, apoptosis, and metastasis including, P2X purinoceptor 7 (P2X7 mucins 4 (MUC4), p53), C-Myb, zinc-finger E-box binding homeobox 1 (ZEB1), EGR2, BR1-associated receptor kinase 1 (BAK1) and SRC kinase signaling inhibitor 1 (SRCIN1) are targeted by miRNA150-5p. Significant downregulation of FOXO4 in C-33A cells expressing miR-150 mimics and the upregulation of FOXO4 (apoptosis factor) in the cells expressing miRNA150-5p inhibitors was also found [45]. The same family of FOX protein was targeted by miRNA27-a as demonstrated by Mozos et al. [35]. FOXO4 transcription arrest is induced by MiRNA150 through binding to mRNA 3′-UTR.Therefore, lowers p27 and pRb activation and increases CyclinD1, finally leading to cell cycle progression and survival. MiRNA150-5p enhances the progression of cell cycle from the G1/G0 to S phase through to decrease of p27 and the increase of CyclinD1 (Zhang et al.) [47]. A common target also for miRNA 27a are cell cycle protein transcription factors [41]. PDCD4 gene which is a direct
suppressor of NF-κB is targeted by miRNA150-5p. It can also suppress AKT pathway as well as the expression of matrix metallopeptidase 9 (MMP-9) which facilitates cancer cell migration [48]. Allgayer et al. have demonstrated that the that the invasion of cells through regulating the expression of urokinase receptor (u-PAR) could be inhibited by PDCD4, which is one of the major invasion-related factors in different cancers [49].

We found a significant association between miRNA 27a and endometrial cell type; it was overexpressed in endometrial cancer type I than type II. Type I EC is usually a well to moderately differentiated cancer and accounts for 80–85% of all ECs and includes tumors of endometrioid histology [50]. A poorly differentiated, usually of a nonendometrioid histological subtype and frequently lacking steroid receptors represents type II. Type I tumors generally arise on a background of endometrial hyperplasia and have a better prognosis. It develops in a steroid environment, associated with high levels of hormone receptors and usually responds to hormonal therapy [51]. While the association between miRNA 27a and EC cell type was evident in our study, the association with tumor grade was borderline, being higher in grade II and III than grade I. No association whatsoever was found with tumor staging denoting that expression in early stages was similar to that in late stages. We thus recommend that that miRNA 27a could be investigated as a biomarker during the post-operative and post-RT follow-up of EC patients in further studies.

In a trial to explain the current study finding miRNA 27a expression in breast cancer was demonstrated as an example [52]. A previous study showed that PR+ versus PR- breast tumors had higher expression of miRNA 27a [53]. This may indicate that miRNA 27a may be regulated by the ovarian steroids estrogen and progesterone in endometrial epithelium. MiRNAs are expressed differentially in the two stages noninvasive (stage I) and myoinvasive adenocarcinomas (stage IB and IC). Overexpressed miRNA 27a was demonstrated in invasive adenocarcinomas, and according to tumor stage, its expression linearly increased. RT-PCR validated the results by in an independent series of EC. The expression of FOXO1 (miRNA 27a main target) was down-regulated in invasive compared with noninvasive tumors. Nonmutated adenocarcinomas showed miRNA 27a overexpression. It was concluded that the tandem of miRNA 27-FOXO1 inhibits apoptosis and enhances tumor cell survival in nonmutated EC [35].

MiR-27a may be used as potential biomarker for patients’ prognoses in cancer. This was demonstrated in different disease conditions. Relapsed lymphocytic leukemia or myeloid leukemia, with high expression level of miR-27a could suggest clinical relapse or poor prognosis. Patients with metastatic or recurrent gastric cancer patients whose miR-27a expression was higher would also lead to poor outcomes. Moreover, liver cancer patients with high miR-27a levels promoted liver cancer proliferation when compared to patients with low miR-27a levels [54].

Recently, miR-27a was demonstrated to act as a key regulator of colorectal carcinoma metabolism favouring an aggressive phenotype and chemoresistance. miR27a exerts pleiotropic effects by modulating diverse and interconnected pathways involved in metabolism reprogramming and associated drug sensitivity. It acts to organize and regulate pathways connecting metabolic rewiring impaired AMPK, activated mTOR, oncogenes crosstalk forced, aerobic glycolysis and chemoresistance in colorectal carcinoma [55].

The current study also demonstrated that miRNA150-5p was overexpressed in postmenopausal more than premenopausal endometrial cancer patients. In postmenopausal women, endometrial cancer predominates. This demonstrates that several extraovarian tissues contribute to the circulating estrogens pool by estrogen production from adrenal steroids in absence of ovarian synthesis. Conjugate estrogens such as E1-S can form estrogen. Sulfatase enzyme, can also produce E1 by the high circulating E1-S metabolite. In malignant endometrium, it can also contribute to estrogen synthesis [56]. In vitro studies using isolated endometrial epithelial and stromal cell cultures have demonstrated the change of miRNAs in uterine tissue by estrogen through their receptor-mediated pathways. In addition to changing the miRNA expression at transcription level. The expression of the miRNA biogenesis can be also influenced by steroids. Among miRNA biogenesis components, Exportin-5 and Dicer1 expressed in the mouse uterus are the major steroid regulated [57]. The expression of p53 targeted by miRNA150-5p, was the highest in adenocarcinoma samples compared to atrophic endometrium and endometrial polyp in postmenopausal women [46].

In the current study miRNA 27a showed 100% sensitivity and specificity, positive and negative predictive values of 100%. MiRNA150-5p showed 88.89% sensitivity and 100% specificity, 100% positive and 78.9% negative predictive values. Areas under the curve were 1.0 for miRNA 27a, 0.982 for miRNA150-5p which were higher than serum Ca 125. The combination of miR27-a and Ca125 had an AUC of 0.894 (95% CI, 0.807, 0.980; sensitivity = 0.774, specificity, 100% positive and 78.9% negative predictive values. Areas under the curve were 1.0 for miRNA 27a, 0.982 for miRNA150-5p which were higher than serum Ca 125. The combination of miR27-a and Ca125 had an AUC of 0.894 (95% CI, 0.807, 0.980; sensitivity = 0.774, specificity = 0.970), as shown in a previous study. Therefore, miRNA 27a can be an optimal non-invasive biomarker to diagnose EC [36]. A recent study identified a series of miRNA/mRNA pairs (miR-23c/DMBX1, miR-670/KCNS1 and miR-497/EMX1) to be associated with survival in EC [58]. Wang (2014) demonstrated similar results; upregulation of miRNA 27a using RT-qPCR in sera of endometroid cancer patients’ sera where AUC was 0.768. Combining miR-27a and CA125 increased it to 0.894 [36].

MiRNA 27a levels in serum and endometrial tumor tissue change in a similar fashion. Gao et al., in 2017 identified
that miR-27a was significantly overexpressed in the tumor tissues and sera of patients with prostate cancer. The relative expression of miR-27a in serum samples of patients who underwent surgery was measured for 3 months to determine whether miR-27a in serum was primarily derived from prostatic cancer tissues. MiR-27a serum levels were significantly less compared to the pre-operative levels, so the increased levels of miR-27a in serum was suggested to originate from cancer tissues in the prostate [59]. Chip hybridization techniques and next-generation sequencing (NGS) compared the expression pattern of miRNA in neoplastic endometrial tissue when compared to hyperplastic tissues or benign. This was validated by RT-qPCR and showed increased expression of miRNA-27a [60].

On the other hand, circulating miRNA150 showed a variable change when compared to tissue. This was demonstrated by Jayaraman (2017) [61] who used fresh frozen paraffin embedded sections (FFPE) and RT-qPCR. Results showed nonsignificant down-regulation of, miRNA-150. The difference between the tissue and circulating miRNA-150 profile may be explained by a cellular selection mechanism hypothesis that peritumor cells secrete miRNA-150 and may act as an important negative feedback regulating agent [62].

In light of the above we suggest a similar diagnostic panel including miRNA 27a and miRNA150-5p to be considered for larger scale studies and further evaluation.

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Data availability Available upon request.

Declarations

Conflict of interest The authors declare there are no conflicts of interest nor financial interests.

Ethical approval The Research Ethics Committee of the Faculty of Medicine, University of Alexandria IRB No 0012098, FWA No 00018699 approved this study and was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Consent to participate All study participants signed informed consents.

Consent for publication All authors approve publication of this version of the work.

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