RESEARCH ARTICLE

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Prognostic Role of Oncogenic and Tumor-Suppressing miRNA Types in Egyptian Uterine Cancer Patients

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Abstract

Objective: Uterine or endometrial cancer affects many women postmenopausal and may reach an advanced stage before signs and symptoms can be noticed. Micro RNAs (miRNAs), non-coding RNAs, play key roles in gene expression regulation and are linked to cancer. This study aimed to elucidate whether some specific types of miRNAs (miRNA133-a, miRNA-21, miRNA-205) can act as prognostic or diagnostic biomarkers for endometrial carcinoma (ER) in Egyptian patients. **Methods:** Blood samples from 36 patients suffering from endometrial carcinoma and 15 healthy volunteers were tested for expression levels of miRNA 133a-2, 21 and 205. **Results:** The expression levels of miRNA133a-2, miRNA-21, and miRNA-205 were significantly elevated in ER patients when compared with the control group, the highest levels were noticed in miRNA133a-2. The CA125 levels were significantly higher in all patients as compared with healthy subjects. **Conclusion:** The findings could support the use of circulating miR133a-2, miR-21 and miR-205 as virtuous prognostic biomarkers for EC in Egyptian patients. The studied miRNA species warrant validation for prospective targeting inhibitory protocols in EC.

Keywords: Endometrial carcinoma- *miR133a-2- miR-21- miR-205*- Egyptian patients

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Introduction

Endometrial carcinoma (EC) affects mostly postmenopausal females. The average age of women diagnosed with endometrial cancer is about 60 years. It is infrequent in women beneath the age of 45. This type of cancer is relatively more public in white women, but it is more fatal to black women and accounts for >76,000 mortality among women each year worldwide (Urick and Bell, 2019). In Egypt, it ranks the 13th most known cancer, and is the third most common gynecological cancer after ovary and cervical cancers, with a high mortality rate and represents 1.6% of total female cancers (Alshahrani et al., 2018). A recent study in Egypt confirmed a low age-standardized rate (ASR) incidence of EC cancer to be 4.1 in 100,000 population (95% CI: 3.8–4.4). The incidence rates have significantly increased over the last 12 years period and the majority of patients (83%) were postmenopausal and diagnosed with adenocarcinoma at early stages (Alshahrani et al., 2019).

The risk factors for EC are categorized to be: women with endometrial overgrowth (hyperplasia), less physical activity, obesity and dietary intake, women who have never had children, menses beginning before age 12, menopause after age 55, estrogen hormonal therapy, tamoxifen intake, radiation to the pelvis, gynecological history, smoking and family history of uterine cancer. Of 127 meta-analyses with cohort studies (Raglan et al., 2019), three connotations were graded with robust evidence. Body mass index and waist to hip relation were linked with amplified cancer danger in premenopausal women. Common signs and indicators of EC are anomalous vaginal blood or discharge, pain during urination and intercourse, and pelvic pain. Uterine cancer is identified typically with a pelvic examination, ultrasound, or biopsy (Faria et al., 2019).

Endometrial cancer is clinicopathologically classified into endometrioid (Type I) and non-endometrioid (Type II, mainly serous) subtypes (Bockman, 1983). Type I EC is moderately or well-differentiated and represents about 80–85% of ECs. It usually grows in an estrogenic atmosphere, surrounded by high levels of hormone receptors, and often give a good response to hormonal remedy. On contrary, Type II EC characterizes almost 15–20% of cancer cases, usually poorly differentiated, mainly of a non-endometrioid histological subcategory, usually deficient with steroid receptors, with a high recurrence rate after therapy, and not reactive to anti-estrogenic treatment; these tumors often grow inside atrophic endometrium (Garikapati et al., 2019).

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On molecular bases, it has been documented that Type I and Types II EC tend to have different mutations involved; inactivation of PTEN occurs early in type I ECs leads to the upregulation of the PI3k/Akt/mTOR pathway, which causes cell growth, but the primary genetic defect in type II EC is a mutation of the p53 gene, observed in 75–100% of tumors (Sherman, 2000). Recently, molecular classification using genomic analysis has allowed for significant advancement in characterization; four unique subgroups have been identified: polymerase ε (POLE) ultra-mutated, microsatellite instability, copy number-low-microsatellite stable, and copy number-high-serious-like. These sub-types have prognostic implications and might support recognizing patients at big recurrence risk at early stages (Winterhoff et al., 2020).

A recent study demonstrated that more than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites suggesting that miRNAs may play an important role in the pathogenesis of a moderate range of human cancers (Pu et al., 2021). The tiny controlling onco-miRNA RNA; microRNA-21 (miR-21) plays a vital role in the overabundance of biological purposes and diseases counting growth, cancer, and inflammation. Recently, other involvements of miR-21 in cardiovascular and pulmonary diseases, including heart and lung fibrosis and myocardial infarction have been designated. Due to the serious roles of its target proteins in many signaling routes, miR-21 has been provoked as an important target for genetic and pharmaceutical treatment in different diseases. Previously, Zhao et al., (2017) estimated that miR-21 promotes cell proliferation and invasion ability while inhibiting cell death in EC through modulating its target gene PTEN at a post-transcriptional level. Thus, we attempted to estimate whether that miR-21 could be a prognostic biomarker or therapeutic target in Egyptian EC patients.

On the other hand, there are three main miR-133 genes in the human genome: miR-133a-1, miR-133a-2, and miR-133b found on chromosomes 18, 20, and 6 respectively. miR-133a-2 has been considered a tumor suppressor and a biomarker for the prognosis of various cancers, such as osteosarcoma, esophageal cancer, colorectal cancer, non-small cell lung cancer, bladder cancer, breast cancer, and gastric cancer (Qu and Li et al., 2020). Wang et al., (2012) also reported that miR-133a-2 is a potential miRNA biomarker and/or regulatory element in circulating monocytes. miR-133a-2 was reported to be dysregulated and negatively correlated with the progress and development of postmenopausal osteoporosis (Wang et al., 2012).

The tumor suppressor microRNA-205 (miR-205) was found to be highly expressed in stem cell-enriched populations from the mouse mammary gland and thus may play a function in normal mammary stem cell maintenance (Greene et al., 2010). Research has shown that miR-205 was significantly down-regulated in prostate cancer compared with match normal tissue. Its re-expression induced apoptosis and cell cycle arrest and resulted in a mesenchymal-to-epithelial transition, such as up-regulation of E-cadherin and reduction of cell locomotion and invasion, and down-regulation of several oncogenes known to be involved in disease progression (Gulei et al., 2018). Several studies have revealed that miR-205 plays important role in the development of gynecological cancers and thus may serve as a potential prognostic biomarker, however, the current conclusions remain controversial. Wu et al., (2020) explored the prognostic significance and functional mechanisms of miR-205 based on a meta-analysis and bioinformatics investigation in a total of 14 published studies containing 5,835 patients. They suggested that miR-205 may be a promising prognostic biomarker and therapeutic target for breast cancer and EC patients.

Therefore, this work aimed to elucidate whether these specific types of miRNAs (such as miR-133a2, miR-21, and miR-205) can act as prognostic biomarkers for Endometrial uterine cancer in Egyptian patients.

Materials and Methods

Subjects' strategy

The present study was carried out on blood samples of 36 patients suffered from endometrial uterine cancer (age range was 50-65 years, mean 57.44±5.01 years and the median age was 57.5 years old) and 15 blood samples of healthy volunteers (age range 50-67 years, mean 58.20±5.17 years and the median age was 58.5 years old) serving as control. Patients' blood samples of endometrial uterine cancer were collected at Damanhour Oncology Center, Damanhour, Elbehera, Egypt. The research protocol was officially permitted by the Committee of Research Ethics, Faculty of Medicine, Tanta University, Code: 34523/2/21). Full medical history was taken with special attention to any associated medical problems. All included patients of endometrial uterine cancer were subjected to full clinical investigations and didn't show clinical symptoms or signs of any other health problems or metastasis. Blood samples from patients undergoing another cancer diagnosis, immune diseases, hepatitis C, or metastasis were excluded. All cancers were histopathologically diagnosed as endometrial adenocarcinoma (EC), 31 cases were of type I, 4 cases were Type II, and all were postmenopausal. Subjects were classified according to the clinical examination into two groups: Group I: 15 Blood samples from normal healthy volunteers (control). All cases in this group were confirmed healthy by clinical examination. Group II: 36 Blood samples of patiewho nts suffered from endometrial uterine cancer with ongoing treatment with chemotherapy.

Blood specimens

Five ml of venous blood were collected from all subjects and were divided into two classes as follows: About 4 ml of total blood was left to clot at 25ºC for 15 minutes, and then centrifuged at 4,000 r.p.m for 10 min. The serum was then separated and divided into several aliquots and stored at -20°C for user determination of different parameters such as iRNA133a, miRNA-21, miRNA-205 expression levels detection, Determination of Cancer Antigen 125 (CA125), Kidney profile: Creatinine and Urea levels Liver Profile: Aspartate aminotransferase (AST) activities, alanine aminotransferases (ALT)

activities. Diabetic Profile: Serum Random Glucose Concentration. Also, one ml of the whole blood samples was separated in a tube with EDTA to perform a complete blood count (CBC).

Isolation and detection of micro-RNA using quantitative Real-Time PCR (qRT- PCR)

Isolation of miRNA

Extraction of Micro-RNA from serum samples of all experiment groups using the Direct-zol™ RNA MiniPrep plus kit (Irvine, CA 92614, U.S.A.) compatible with TRI Reagent® (Catalog no. R2050). Samples were lysed and homogenized in the Lysis Buffer of TRI Reagent, which contains phenol and guanidine thiocyanate in mono-phase solution to facilitate the immediate and most effective inhibition of RNase activity. Complete removal of DNA was performed by using DNase I digestion. miRNA purification and concentration were detected by nanodrop (A260/A280 >1.8, A260/A230 >1.8). The obtained miRNA was stored at -80°C until use.

cDNA preparation

DNA (cDNA) single-stranded complementary DNA was obtained from 1µg of purified miRNA using the SensiFAST™ cDNA Synthesis Kit (Bioline, Catalog no. BIO- 65053) according to the manufacturer's manual.

SYBR green RT-PCR assay

All PCR runs were performed on the Applied Biosystems Step One™ Instrument. Samples belonging to the same group were always run together to prevent any inter-run variation. To determine the optimal concentration of primers and SYBR Green RT-PCR reactants and conditions, preliminary tests were performed before the main experiment. Real-time PCR reactions were performed using Hot Start Taq DNA Polymerase a modified form of QIAGEN Taq DNA Polymerase.

Analysis of qRT-PCR data

In all patient groups, the CT cycle (Threshold cycle) was employed to determine the expression level. Using Applied Biosystems Step OneTM Instrument software, the miRNA expression level was determined as described by Yuan et al., (2016). Using the following formula, the results were expressed as the ratio of reference gene to target gene: ΔCT (cycle figures at the threshold data of log-grounded fluorescence normalized to β-actin) = CT (target genes) – CT (β-actin). The following formula was used to calculate the relative expression levels ΔΔCT = ΔCT (patients) – ΔCT (β-actin). Therefore, the expression levels were presented as n-fold changes in the calibrator (RQ; qReal-time PCR). The value was used to scheme the expression of the devoted genes using the expression of $2^{-\Delta\Delta CT}$ (Schmittgen and Livak, 2008). All samples from patients and control subjects were analyzed twice for confirmation.

In vitro assay for the quantitative determination of CA125

The quantitative determination of CA125 antigen in serum of all patients and control subjects was performed by CA125 II™ family collection assay on the LIAISON® Analyzer (LIAISON® DiaSorin, Italy). The technique is a sandwich chemiluminescence immunoassay using two different highly specific monoclonal antibodies for the coating of the solid phase (magnetic particles) and the conjugate (Szymańska et al., 2020). Briefly, during the first incubation, CA125 present in calibrators, patients or controls binds to the solid phase monoclonal antibody, and subsequently, after a washing step in the second incubation, the antibody conjugate reacts with CA125 already bound to the solid phase. After incubation, the unbound material is removed with a wash cycle. The amount of isoluminol-antibody conjugate is measured by a photomultiplier as relative light units and is indicative of CA125 concentration present in calibrators, samples, or controls.

Determination of complete blood count and serum glucose level

The complete Blood Count Assay was performed by the Full Automated CBC counter URIT-3300 Serial No. E1122, China. Blood glucose levels (the subjects were 8 to 12 hours fasting before analysis), and renal and liver functions tests were assayed by using commercial kits supplied by Spectrum Diagnostics, Hannover- Germany (www.Spectrum-diagnostics.com) according to the manufacturer's manuals.

Statistical analyses

IBM SPSS software, USA, ver. 18.0 was used for statistical analysis. One-way analysis of variance (ANOVA) and χ^2 test were used to analyze the significance between groups. Statistical significance was determined at $P<0.05$.

Results

Patients' characteristics

Age distribution

The age distribution in group I (control) showed that the mean age was 57.44±5.01 years (range 50-65 years, median age 57.5 years) and the mean age in group 2 (patients) was 58.20±5.17 years (range 50-67, median age 58.5 years). There were no significant differences between the age in the two groups ($P>0.05$).

Blood picture, random blood glucose levels, liver and kidney enzymes levels

Table 2 shows a comparison between the two studied groups regarding blood parameters, random glucose levels as well as kidney and liver functions. The hemoglobin level showed a statistically significant decrease in patients below the control; 10.1±0.08 g/dl vs.12.0 ± 0.91 g/dl respectively, and the total numbers of WBCs were significantly higher in patients than in the control. The platelet count was significantly lower in patients than in the control ($P<0.05$). Data for Random Blood Glucose (mg/dl.) were incomparable between the two groups (Table 2).

The creatinine levels in control individuals and patients were almost similar (0.70±0.02 and 0.81±0.03 respectively), while the Urea levels in patients were found

Table 1. Primer Sequences Used in the Study

Gene	Primer Sequence (5′–3′)
GAPDH gene	Forward 5`-ATGGAGAAGGCTGGGGCTCACCT -3`
	Reverse 5`-AGCCCTTCCACGATGCCAAAGTTGT -3`
miR-133a	Forward 5-GGAGCCAAATGCTTTGCTAGA-3′
	Reverse 5-CGCCATCAATGCACAGCTAC-3′
miR-21	Forward 5′-GCCCGCTAGCTTATCAGACTGATG-3′
	Reverse 5′-GTGCAGGGTCCGAGGT-3′
miR-205	Forward 5-ATCCTCAGACAATCCATGTGCT-3′
	Reverse 5-ACTCCACTGAAATCTGGTTGGG-3′

All primer sequences were obtained from the Gene Bank of NCBI BLAST table.

Table 2. Blood Biochemistry Data for Random Glucose Levels, Kidney and Liver Enzymes Activities in Control Subjects and Patients

Blood & Serum Biochemical Parameters	Control (n=15)	Patients (n=36)
HB% (g/dl)	12.1 ± 0.91	10.1 ± 0.08*
Total WBCs (X1,000/µl)	5.5 ± 0.20	9.0 ± 0.29*
Platelets count X1,000/ul	297.1 ± 7.8	179.4 ± 4.15*
Random Blood Glucose (mg/dl.)	84.2 ± 10.5	86.8 ± 11.7
Creatinine (mg/dl.)	0.70 ± 0.02[a]	0.81 ± 0.03
Urea (mg/dl.)	25.4 ± 2.49	35.3 ± 3.60
ALT (U/L) (mg/dl.)	13.3 ± 2.33	35.0 ± 3.70*
AST (U/L) (mg/dl.)	13.7 ± 2.26	34.5 ± 4.65*

a, All values are means ± S.D; HB, Hemoglobin; WBC, White blood Cells; AST, Aspartate aminotransferase; ALT, alanine aminotransferases; *Significant vs. control group at P<0.05.

to increase (35.3±3.60) over the controls (25.4±2.49), both were in the normal range. Also, the activity levels of the liver enzymes ALT and AST in patients were significantly increased than in controls, (P<0.05), however, all levels of kidney and liver function enzymes for patients and control are within the normal clinical range (Table 2).

Cancer Antigen 125 (CA125) levels in Serum

The average serum levels of CA125 in control was 13.0±1.36 and in patients was 73.1±5.38 U/ml, there was a highly significant increase in CA125 in patients more than in the control group (P<0.001) (Figure 1).

Quantitative miRNA gene expression analysis

The miRNA expression of miR133a-2, mi-R21, and miR-205 analysis raw data, as well as data normalized according to RT-PCR cycle threshold or relative to GAPDH housekeeping endogenous control gene, are summarized in Table 3. The average normalized expression (RQ) of miR133a-2 was found significantly elevated in patients of group 2 by about 12- fold as compared with the normalized normal control miRNA expression in GAPDH. Also, the relative expression of miR-21 is found significantly elevated in patients' sera by about 7-fold as compared with RQ levels of normalized controls. Moreover, the miR-205 has also shown about 4-fold overexpression above controls normalized with the endogenous housekeeping control (P<0.05) (Figure 2). Figures 3,4 show log curves of microRNA expressions in qRT-PCR raw and quantitation data in Cycling A. Green relative to GAPDH.

Table 3. qRT-PCR Data Analysis of *miR133a-2* Relative to *GAPDH* Endogenous Gene

Groups	*GAPDH* CT	*miR133a-2* CT	$^{\Delta}$CT	$^{\Delta\Delta}$CT	RQ $^{2-\Delta\Delta}$CT
Norm Control	23.01±0.12	31.34±1.28	8.33	0	1.00± 0.00
Patients Group	25.47±1.38	30.24±2.41	4.77	-3.56	11.7942±1.81*

a, Each value is signified as the average of tasters in triplicates; b, RQ Values are means ± S.D. CT, Cycle threshold; $^{\Delta}$CT, Cycle threshold of miRNA – Cycle threshold of GAPDH; $^{\Delta\Delta}$CT, $^{\Delta}$CT - $^{\Delta}$CT (Normal subjects); RQ, Real-time qPCR; *, Significant versus. G1 at P<0.05. S.D. for RQ data is for the triplicate repetition of analysis.

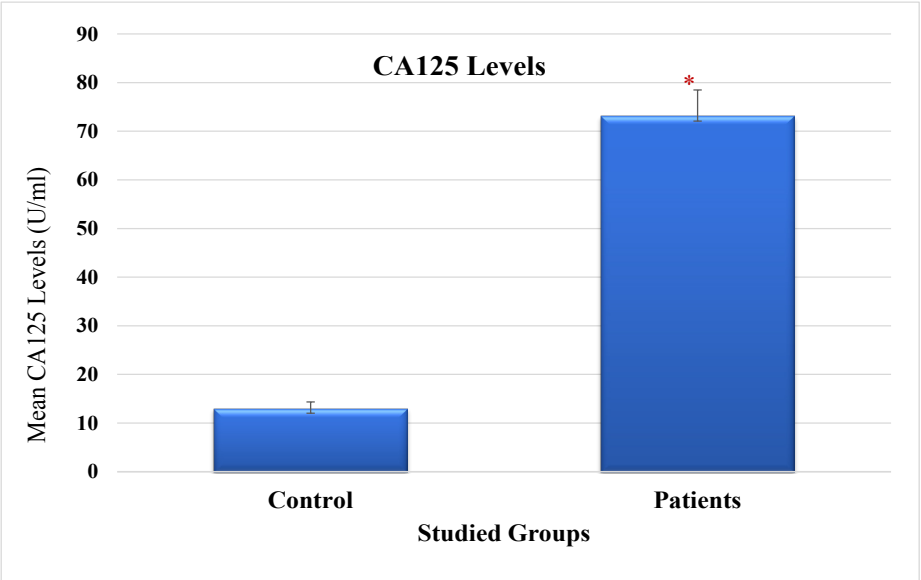

Figure 1. Comparison between Control and Patient Groups Regarding CA125 Levels. *: P<0.05 vs. control group.

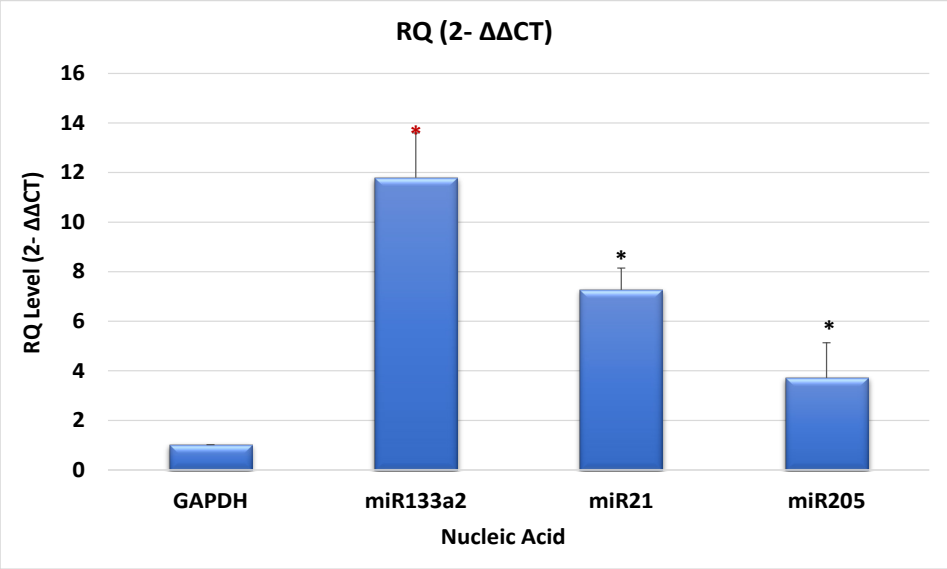

Figure 2. Average qRT-PCR Analysis Data of the Studied *miRNAs* Normalized Relative to *GAPDH* Endogenous Housekeeping Gene. *: Significant vs. GAPDH RQ at P<0.05.

Table 4. qRT-PCR Data Analysis of *miR-21* Relative to *GAPDH* Endogenous Gene

Groups	*GAPDH* CT	*miR-21* CT	$^{\Delta}$CT	$^{\Delta\Delta}$CT	RQ $^{2-\Delta\Delta}$CT
Norm Control	23.01±0.12	23.42±0.20	0.41	0	1.00± 0.00
Patients Group	25.47±1.38	23.02±0.69	-2.45	-2.86	7.26015±0.89*

a, Each value is signified as the average of tasters in triplicates; b, RQ Values are means ± S.D. CT, Cycle threshold; ^{A}CT, Cycle threshold of miRNA – Cycle threshold of *GAPDH;* $^{\Delta\Delta}$CT, $^{\Delta}$CT - $^{\Delta}$CT (Normal subjects); RQ, Real-time qPCR; *, Significant versus. G1 at P<0.05. S.D. for RQ data is for the triplicate repetition of analysis.

Discussion

The present work aimed to explore the significant expression levels of miRNA133a-2, miR-21, and miR-205 in Egyptian EC patients. In the present study, the ages ranged from 50 years to 65 in patients and 67 years

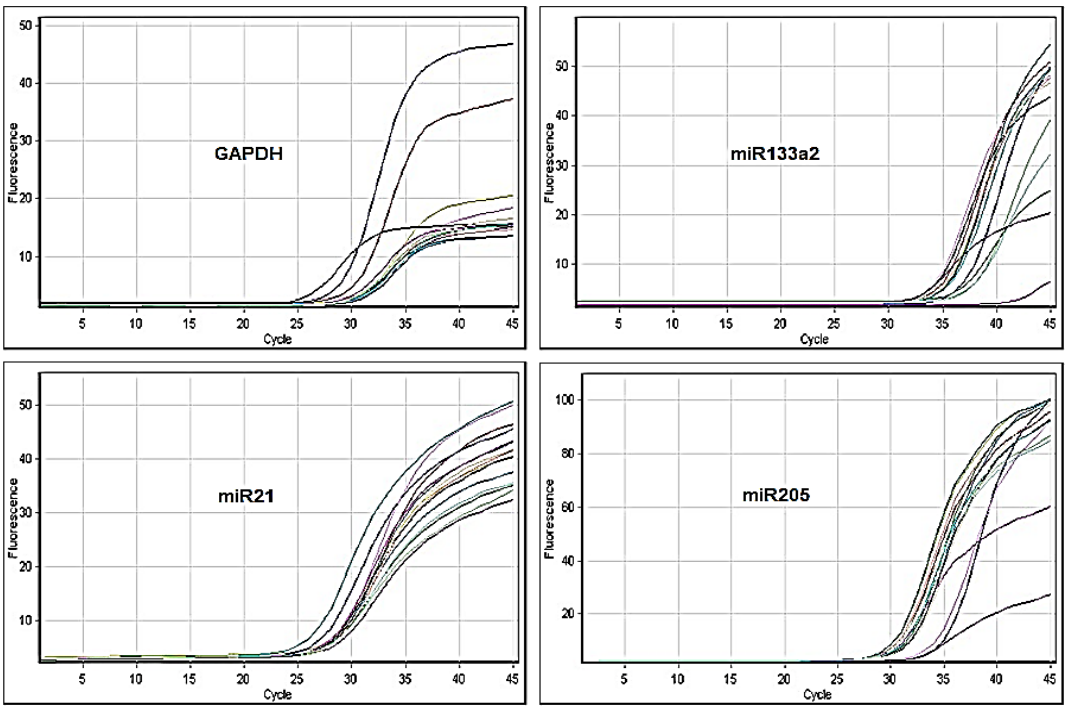

Figure 3. Log Curves of qRT-PCR Raw Data in Cycling A. Green for microRNA expressions relative to *GAPDH* housekeeping gene. Upper left curves are for the *GAPDH* housekeeping gene; Upper right curves are for *miR133a-2*; Lower left curves are for *miR-21*; Lower right curves are for *miR-205*.

Table 5. qRT-PCR Data Analysis of *miR-205* Relative to *GAPDH* Endogenous Gene

Groups	*GAPDH* CT	*miR-205* CT	$^{\Delta}$CT	$^{\Delta\Delta}$CT	RQ $^{2-\Delta\Delta}$CT
Norm Control	23.01±0.12	25.24±0.57	2.23	0	1.00± 0.00
Patients Group	25.47±1.38	25.81±1.62	0.34	-1.89	3.70635±1.42*

a, Each value is signified as the average of tasters in triplicates; b, RQ Values are means ± S.D. CT, Cycle threshold; $^{\Delta}$CT, Cycle threshold of miRNA – Cycle threshold of *GAPDH*; $^{\Delta\Delta}$CT, $^{\Delta}$CT - $^{\Delta}$CT (Normal subjects); RQ, Real-time qPCR; *, Significant versus. G1 at P<0.05. S.D. for RQ data is for the triplicate repetition of analysis.

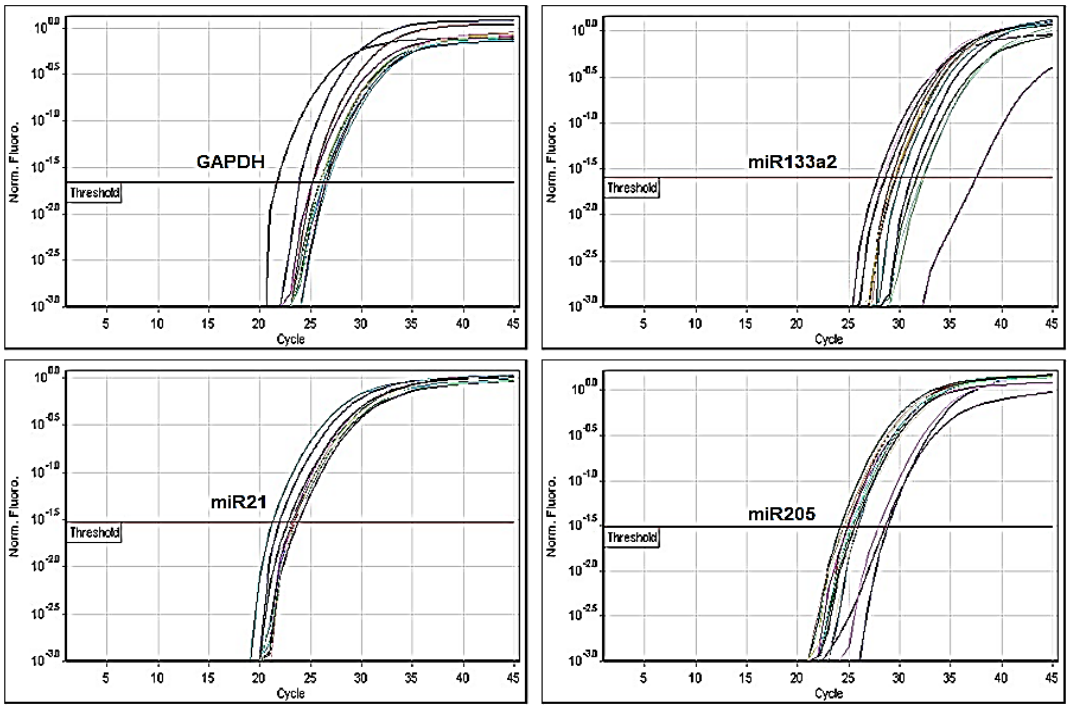

Figure 4. Representative Log Curves of qRT-PCR Quantitation Data in Cycling A. Green for microRNA expressions relative to *GAPDH* housekeeping gene. Upper left curves are for the *GAPDH* housekeeping gene; Upper right curves are for *miR133a-2*; Lower left curves are for *miR-21*; Lower right curves are for *miR-205*.

in normal control subjects. The statistical comparison showed no significant differences regarding average or median ages between the two groups (P>0.05), these criteria were central to abolishing the effect of age on the present net results, and agree with the inclusion criteria resulting in nearly homogenous groups (Patino and Ferreira, 2018).

The comparison of blood CBC data investigated between patients and healthy subjects showed significant differences in Hb, WBCs, and blood platelet levels between both groups. Despite these differences, possibly due to the effect of the disease and more possibly after chemotherapy, all the data are in the normal range. In agreement with this finding, a previous study indicated a significant decrease in Hb level & platelet count in EC Patients (Shen et al., 2017). Moreover, the same case occurred in liver and kidney function tests as significant differences were observed in the data was noticed between the two studied groups, again, all the data were within the normal range. These results are in agreement with a study carried out by Cochrane et al., (2019), who showed no changes in kidney or liver functions in EC patients, but these levels were significantly changed when metastasis occurred in both organs. Also, the random blood glucose in both studied groups was in the normal range, probably due to the absence of metastasis in the pancreas.

Furthermore, the level of CA125, the EC marker in serum showed about 5.6 folds increase in patients when compared with corresponding values in normal healthy volunteers. Previous data suggested that CA125 levels are beneficial in managing and follow-up patients with EC. Increased CA125 levels are not only linked with EC or metastasis but also associated with recurrence (Yildiz et al., 2012). It seems that the threshold cut-off level for a normal serum CA125 in patients with EC is lower than the customarily recognized threshold value of 35 U/mL, as estimated for other clinical sceneries such as ovarian cancer (Yildiz et al., 2012; Povolotskaya et al., 2014). In 1994, it was recommended that a lesser threshold of CA125 would be suitable for validation in EC (Alagoz et al., 1994). Recently, it was postulated that a cut-off value of 20U/mL may be the proper threshold to classify excess uterine blowout whereas other investigations have used the cutoff of 35U/mL (Kim et al., 2010). Regrettably, there is no investigation with a huge number of contributors and no putative threshold value of CA125 to base apply on. The analysis in this study suggests adopting a threshold level of 28U/mL. This is consistent and within the range of the CA125 threshold levels recommended by Kim et al., (2010). In another recent study by Nazarian et al., (2019),

the CEA mRNA biomarker was found to be positive for most of the participants in sick patients group with oral squamous cell carcinomas and as compared with the people in the healthy group.

Since their detection, miRNAs appeared as significant molecules during cancer initiation, pathogenesis, and progression. They have also been recommended as effectual indicators in cancer diagnostics and conclusion estimates (Farazi et al., 2011). Several studies are previously and presently conducted exploring treatment options associated with modulations of miRNA levels in vitro and in vivo and for its use as target molecules for therapy (Meyer et al., 2010; Karkhane et al., 2020). As single-stranded miRNAs are understood to control hundreds of genes, suitable regularization of miRNA studies may have a lot greater influence on the biological application of developed data about mRNA expression examination (Karkhane et al., 2020). As endometrial carcinoma (EC) is classified into two subtypes; Type-I and Type-II, while type-I ECs are classified according to cytological inspections into 3 main grades (G1, G2, and G3) (Tsikouras et al., 2013), the analysis of surrogate markers such as; POLE, MSI, and p53 and other validated molecular alterations (L1CAM or miRNAs) (Winterhoff et al., 2020), leads to the possibility to acquire a cohesive molecular risk outline. Ongoing studies are utilizing this risk profile to identify patients who may benefit from early diagnosis or additional treatment for early-stage by the use of surrogate endpoint markers such as miRNAs (Ventz et al., 2021).

Here, the normalized miRNA133a-2 relative to the endogenous housekeeping gene GAPDH, show a significant increase in patients more than the normal subjects' expressions; the fold change was about 12-fold. In line with this, Yamamoto et al., (2015) demonstrated that regulation of the miR133a-2 enhanced cancer cell migration and invasion through the overexpression of phosphor di esterase-7A (PDE-7A) in EC cells. Prediction of miRNA targets revealed that PDE-7A was a possible target gene controlled by miR133a-2. This warrants exploration.

Also, the miRNA-21 showed a significant increase in patients' sera more than the normalized control group, the fold change in patients was about 7-folds. A large number of studies on miRNAs have been published. Many efforts have been made to translate the findings into clinical practice, such as miravirsen's inhibition of miR-122 in the treatment of hepatitis C (Janssen et al., 2014). Although miRNAs have been shown to play a role in the development, diagnosis, and prognosis of lung cancer, clinical trial outcomes have not been encouraging in terms of EC cancer treatment. More research is needed to determine whether or not miRNA-focused therapy can assist EC cancer patients. In a meta-analysis study (Gao et al., 2016), the expression values of serum miR-21 were considerably greater in benign lesion patients and EC patients compared to healthy controls. When compared to patients with benign lesions, EC patients had greater levels of miR-21 expression. Finally, the meta-analysis showed that circulating miR-21 has great diagnostic performance for a variety of malignancies, and the validation test shows that serum miR-21 could be used as a new biomarker for endometrial carcinoma, in line with the present results.

On the other hand, the miRNA-205 showed significantly increased expression levels in patients than in the GAPDH-normalized normal control subjects; the mean fold change was about 4-folds. It was previously postulated that the expression levels of miR-205 were significantly increased in EC patients in the New York area- USA as compared to normal tissues with a decreased expression of a miR-205 target PTEN detected in EC tissues compared to normal tissues (Karaayvaz et al., 2012). In that study, Kaplan-Meier survival analysis revealed that high levels of miR-205 expression were associated with poor patient overall survival (hazard ratio, 0.377; Log-rank test, P = 0.028) revealing that miR-205 holds a unique potential as a prognostic biomarker in EC (Karaayvaz et al., 2012).

To the best of our knowledge, the functional role of miR-205 in endometrial cancer has not been fully discovered. Zhang et al., (2014) have previously suggested that it could act by inhibiting phosphatase and tensing homolog protein, while Su et al., (2013) found that miR-205 promoted tumor proliferation and invasion through targeting estrogen-related receptor gamma. Torres et al., (2016) hypothesized that inhibition of miR-205 would decrease the growth of EC. Interestingly, the in vitro and in vivo inhibition of miR-205 by locked nucleic acid (LNA)-modified antisense oligonucleotides (a miR-205 inhibitor) works by limiting the proliferation of EC cells. On the other hand, binding and inhibiting microRNA 205 5p function and indirectly increasing PTEN activity by long non-coding RNA (lncRNA) LA16c 313D11.11 was also shown recently to modulate the progress of EC (Xin et al., 2020).

Lotvall and Valadi et al., (2007) have reported the early results of extracellular miRNAs in exosomes. Exosomes are extracellular vesicles and miRNA carriers are released from cells into the blood. The miRNAs are bundled inside exosomes (exosomal-shuttle RNA (esRNA)); RNA binding proteins (RBPs) take esRNA into exosomes. Statello et al., (2018) assumed that 30 RBPs make complexes with miRNAs and transport them into exosomes during the biosynthesis of exosomes forming RNA–RBP complexes with the cellular RNA and exosomal RNA types.

Catabolic enzymes for miRNA exist in serum and it was previously believed that miRNAs might not occur in the blood. Though, miRNAs in exosomes are steady and are existing in the blood (Kameshwar et al., 2020). Exosomes unconstrained from cancer cells probably are related to other pathways including immune suppression, drug resistance, or angiogenesis. Accordingly, miRNAs in exosomes are similar to cell characteristics as overexpressed miRNAs in cancer cells are included in exosomes released from these cells. Subsequently, the development of biomarkers using miRNAs has increased (Condrat et al., 2020).

Numerous miRNAs have precise expression outlines in certain carcinomas and are expected to be valuable as biomarkers. Examination of miRNAs may categorize cancer more appropriately than the examination of

extremely huge numbers of mRNAs within the cells. Lung cancer with downregulation of let-7miRNA has a poor prognosis after surgery, indicating that miRNAs may be markers for prognosis, as well as for early diagnosis (Chin et al., 2008). Therefore, the findings in the present study indicate that the three studied miRNAs (miR133a-2, miR-21, and miR-205) in EC in Egyptian patients could support their use as good prognostic biomarkers for EC. Many studies are warranted to validate different miRNA inhibitory protocols for targeting the studied miRNA in EC.

Author Contribution Statement

E Salim set the conception and the experimental design, was a major contributor to writing the manuscript, and performed the molecular analyses and data interpretation; D Beltagy contributed to molecular analysis and contributed to revising the manuscript. N Elmashad has planned and revised the physiological and pathological analysis. M Abodonia has collected the samples, contributed to the physiological analysis, performed statistics, and contributed to writing the manuscript.

All authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study); AND to have agreed both be personally accountable for the author's contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors read and approved the final manuscript.

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The ethical committee that approved the research

The research protocol was officially permitted by the Committee of Research Ethics, Faculty of Medicine, Tanta University (Code: 34523/2/21).

Availability of data (if applicable to your research)

All data generated or analyzed during this study are included in this published article.

Conflict of interest

The authors declare that they have no competing interests and no conflict of interest with any governmental or non-governmental parties.

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