Mir-21 and Mir-125b as theranostic biomarkers for epithelial ovarian cancer in Tunisian women

A Habel[1], F Nassar[2], M Itani [3], H Bouaziz [4], M Hadj-Ahmed[1], Z Msheik[3], M Stayoussef[1], R Nasr[3], B Yacoubi-Loueslati[1]

1. Laboratory of Mycology, Pathologies and Biomarkers (LR16 ES05), Faculty of Sciences of Tunis, University Tunis El Manar, Tunis, Tunisia.
2. Department of Internal Medicine, Naef K. basile Cancer Institute American University of Beirut, Beirut, Lebanon.
3. Department of Anatomy, Cell Biology and Physiological Sciences, American University of Beirut, Beirut, Lebanon.
4. Department of Carcinological Surgery, Salah Azaiez Institute, Tunis.

Abstract

Background: Ovarian cancer (OC) is the third most common cancer in women and the leading cause of death associated with gynecologic tumors. Because this disease is asymptomatic in the early stages, most patients are not diagnosed until the late stages. This highlights the need for the development of diagnostic biomarkers. MicroRNAs (miRNAs), small non-coding RNAs, are currently being explored as potential biomarkers for the early detection of various malignancies in humans. However, their expression and diagnostic value in OC have not been well studied.

Materials and Methods: the plasma levels of miR-21, miR-200a, miR-200b, miR-200c, miR-205 and miR-125b were determined in epithelial ovarian cancer (EOC) patients and healthy controls by Reverse Transcription Quantitative Realtime Polymerase Chain Reaction *(RT-qPCR)*. The expression levels of the deregulated microRNAs were analysed according to clinical characteristics.

Results: It was found that miR-21 and miR-125b were upregulated in EOC compared with healthy controls. Moreover, decreased miR-125b was associated with resistance to platinum-based chemotherapy.

Conclusions: Our data suggest that miR-21 and miR-125b in plasma may serve as potential circulating biomarkers for the early detection of EOC. MiR-125b may also be useful for predicting chemosensitivity in EOC patients.

Keywords: Epithelial ovarian cancer; miRNAs; biomarkers; resistance to treatment; Tunisia.

DOI: https://dx.doi.org/10.4314/ahs.v23i2.29

Cite as: *Habel A, Nassar F, Itani M, Bouaziz H, Hadj-Ahmed M, Msheik Z, et al. Mir-21 and Mir-125b as theranostic biomarkers for epithelial ovarian cancer in Tunisian women. Afri Health Sci. 2023;23(2):256-64. https://dx.doi.org/10.4314/ahs.v23i2.29*

Introduction

Epithelial ovarian cancer (EOC) is the major form of ovarian cancer (OC) that severely threatens women's health worldwide. It has the highest mortality rate among all gynecologic cancers [1, 2]. This is because it is not easily diagnosed at an early stage. EOC is often referred to as a 'silent killer' because it is a tumour that spreads in the abdomen and metastasizes without specific symptoms. Therefore, most EOC patients are not diagnosed until an advanced stage [2]. Recent reports indicate that 80% of patients with advanced EOC have an inferior prognosis and relapse [3]. To date, clinical treatment of EOC has been based on cytoreductive tumour surgery and a combination of paclitaxel and platinum-based chemotherapy. Unfortunately, EOC has both a high recurrence rate and drug resistance which causes a high mortality rate [4,5]. Hence, to improve the survival rate of EOC patients, there is an urgent need to identify new diagnostic and theranostic biomarkers characterized by higher specificity and sensitivity.

Recently, microRNAs (miRNAs) have been analysed as potential biomarkers for the early detection of various types of cancer, including EOC [6,7]. miRNAs were discovered as a new class of evolutionarily conserved small non-coding molecules [8]. They play an important regulato-

Corresponding author:
Azza Habel,
Laboratory of Mycology, Pathologies and Biomarkers (LR16 ES05), Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia
Email: azza.habel1@gmail.com

ry role in cellular homeostasis [9]. They also control normal cell development, differentiation, and apoptosis and determine cancer cells' final phenotype [10]. Several miRNAs are dysregulated in EOC 7. Some studies have reported that some miRNAs such as miR-15, miR-31, miR-155, miR-127, and miR-99b are downregulated in EOC compared with controls, suggesting that they function as tumour suppressors [11-13]. Other miRNAs such as miR-199a, miR-200a, miR-200b, miR-200c, miR-21 and miR-125b are overexpressed in EOC and may be considered cancer promoters or oncomiRs [14,15]. However, few studies have investigated the potential value of miRs as theranostic biomarkers for EOC.

In this regard, we analysed a panel of miRNAs: miR-21, miR-200a, miR-200b, miR-200c, miR-205, and miR-125b to evaluate their potential diagnostic and theranostic value in EOC.

Materials and Methods

Study subjects

Institutional review board (IRB) approval for this study was first obtained from the Salah Azaeiz Institute of Tunis (SAI) Research and Ethics committee (IRB reference: 01/ISA/2019). A total of 49 EOC patients (mean age = 52.47 ±12.72). All were newly diagnosed outpatients at the SAI surgical and oncology service between June 2019 and November 2020. All patients received no therapy at the time of recruitment and were followed for at least 6 months after treatment using their medical records. This was done to determine whether or not they responded to treatment or had a relapse.

Twenty-five age-matched healthy women who had no personal or family history of EOC or any type of cancer were included as healthy control subjects. None of the subjects considered (cases and controls) had any unrelated comorbidity. The clinicopathological characteristics of the patients were obtained from medical records and interviews using a structured questionnaire. Patients' medical records were also reviewed 6 months after sampling to monitor response to chemotherapy (CT). All patients and controls provided written informed consent.

Blood sampling

Five millilitres of venous blood were collected in a sterile tube containing EDTA. Plasma samples were separated within 2 hours of collection using a ficoll gradient centrifuging at 2.500 rpm for 20 minutes. Next, plasma samples were transferred to a clean microcentrifuge tube and centrifuged at 14.000 rpm for 10 minutes to remove cell debris and fragments. Finally, plasma samples were aliquoted and stored.

RNA extraction

RNA extraction and cDNA synthesis

Total RNA (including miRNA) was isolated from 250µl of plasma using the Plasma/Serum Circulating and Exosomal RNA Purification Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions. The quantity and quality of the extracted RNA were determined using the DeNovix DS-11 FX spectrophotometer (Wilmington, DE, USA). For cDNA synthesis, reverse transcription of ten nanograms of the total RNA was performed using the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's instructions [16,17]. The reaction mixture was incubated at 16°C for 30 minutes, 42°C for 30 minutes, and 85°C for 5 minutes. Finally, the cDNA samples were diluted in 57µl DEPC water.

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

RT-qPCR for miR-21, miR-200a, miR-200b, miR-200c, miR-205, and miR-125b was performed using validated primers and probes from the TaqMan® microRNA Assays Kit, and 2x TaqMan® Universal Master Mix without Amperase Uracil N-glycosylase (UNG) (Applied Biosystems, Waltham, MA, USA). The reaction mix was incubated for 10 minutes at 95°C and 40 cycles of 10 seconds at 95°C and 60 seconds at 60°C using Bio-Rad CFX96 Real-Time System, C1000 Thermal Cycler (Hercules, CA, USA). The RT-qPCR reaction was performed in duplicate for miR-21, miR-200a, miR-200b, miR-200c, miR-205 and miR-125b. MiR-16 was used as an endogenous control. We calculated relative miR expression (fold change expression) using the equation 2^-ΔΔCt: ΔΔCt=ΔCt (EOC samples)-ΔCt (control samples) and ΔCt = Cq target (miR of interest)- Cq (miR-16) [18].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 and a p-value < 0.05 was considered statistically significant. Data are presented as mean ±SD. Differences between groups (EOC patients and controls) were compared using the Fold Change and Wilcoxon's Signed Rank

test. Differences between subgroups of patients were determined using the Mann-Whitney- test or the Kruskal-Wallis's test.

Results

Study subject

Clinical and pathological data of 49 Tunisian EOC patients are summarized in table 1. Their mean age was (52.47±12.72). The mean body mass index (BMI) is (27.66±4.484) and 22.4% of patients were obese. According to the International Federation of Gynecology and Obstetrics (FIGO), 14 (28.5 %) patients were diagnosed with early-stage EOC (stage I and stage II), 35 (71.5%) patients were diagnosed with late-stage EOC (stage III and stage IV), and 19 (38.78%) patients had distant metastasis. All patients were treated with six doses of a combination of Taxol and carboplatin CT that can be used as an adjuvant (before surgery) or after surgery. This depends on the size, grade, and type of tumour. Nine (18.36%) cases were sensitive to chemotherapy (Taxol-Carbo) and fourteen recurred after six months of treatment (Table 1).

Table 1. Characteristics of study participants

		Cases n=49 (%)	Controls n=25 (%)	p
Age (years)[1] (mean ±SD)		52.47±12.72	52.14±18.37	0.3914
BMI (kg/m²)[1] (means ±SD)		27.66±4.484	26.48±6.829	0.5602
Menarche (years) (mean ±SD)		12.49±1.218	-	-
Nulliparous[2]	nulliparous	18 (36.73)	-	-
	Uniparous	8 (16.32)		
	Multiparous	23 (46.93)		
Menopausal status[1]	Pre-menopausal	18 (32.8)	-	-
	Post-menopausal	31 (67.2)		
Users of oral contraception[1]	No	40 (81.6)	-	-
	Yes	9 (18.4)		
FIGO staging[1]	Early stage (I+II)	14 (28.5)	NA	NA
	Late stage (III + IV)	35 (71.5)		
Grade[1]	Low grade (I+ II)	28 (57.14)	NA	NA
	High grade (III)	21 (21.86)		
Recurrence of disease[1]	No	30 (61.22)	NA	NA
	Yes	19 (38.78)		
Distant metastasis [1]	No	30 (61.22)	NA	NA
	Yes	19 (38.78)		
CA-125	<35 (U/ml)	10 (20.4)	-	-
	>35 (U/ml)	30 (61.2)		
	ND	9 (18.4)		
Response to chemotherapy[1]	Sensitive	40 (81.64)	NA	NA
	Resistant	9 (18.36)		

SD: standard deviation; BMI: Body Mass Index; FIGO International Federation of Gynaecology and Obstetrics; [1] p value performed with Mann-Whitney test; [2] performed with Kruskal-Wallis test; NA: Not applicable; ND: not determined

Expression levels of miR-21 and miR-125b in plasma EOC patients and controls

The expression levels of miR-21, miR-200a, miR-200b, miR-200c, miR-205, and miR-125b were measured in the plasma of 49 newly diagnosed EOC patients and compared with their expression in the plasma of 25 healthy individuals. The expression levels of miR-21 and miR-125b in plasma were significantly higher in EOC patients compared to healthy controls (p=0.0038*; p=0.0005*, respectively) (figure 1). On the other hand, miR-200a, miR-200b, miR-200c, and miR-205 were not significantly dysregulated in the plasma of EOC patients (data not shown).

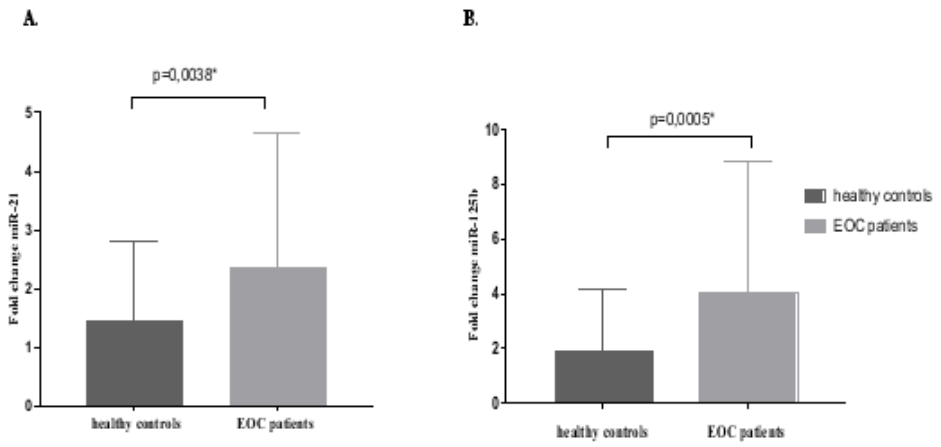

Figure 1: Upregulated miRNA expression in Tunisian Ovarian cancer plasma.

Association between miR-21 and miR-125b and the pathological features of EOC

To better assess the possible association of miR-21 and miR-125b with the development of EOC, fold changes of these two miRs were compared between EOC patients stratified according to different clinicopathological characteristics such as age, BMI, nulliparity, menarche, menopause, oral contraception, FIGO staging, grade, disease recurrence, distant metastasis, and response to treatment. The results did not reveal any significant association between age, BMI, menarche, menopause, FIGO staging, grade, disease recurrence, distant metastasis and miRs plasma levels (Table 2). However, we observed that the expression of miR-21 significantly increased in multiparous women (p= 0.0439*) and women who took birth control pills (p= 0.0315*). In addition, the level of miR-125b was significantly higher in EOC patients who responded to chemotherapy (carbo-Taxol) than in those who developed resistance to this CT regimen (7.241 ±7.117 vs. 2.761 ±3.5; p=0.0457*) (Table 2).

Table 2: Comparison between relative expression of miR-21 and miR-125b and clinicopathological parameters

		miR-21		miR-125b	
		Fold change (means±SD)	**p**	**Fold change (means±SD)**	**p**
Age (years)[1]	≤52	3.889±3.849	0.4887	5.59±6.082	0.9507
	>52	3.455±3.81		5.72±7.197	
BMI (kg/m²)[1]	Underweight + normal	2.635±3.044	0.1040	3.358±4.566	0.1267
	Overweight + obese	4.402±4.085		6.755±7.033	
Menarche (years)[2]	>12	3.766 ±3.654	0.9563	1.267 ±1.227	0.051
	12-13	3.483 ±3.68		5.551 ±6.343	
	<14	3.93 ±4.408		8.253 ±7.702	
Nulliparitous	nulliparous	3.059 ±3.907	0.0439*	3.796 ±4.964	0.1643
	Uniparous	1.27 ±1.15		6.929 ±9.609	
	Multiparous	4.81 ±3.897		6.455 ±6.314	
Menopausal status[1]	Pre-menopausal	2.281 ±2.49	0.1240	3.165 ±3.707	0.1251
	Post-menopausal	4.473 ±4.211		7.21 ±7.496	
Users of oral contraception [1]	No	3.276 ±3.582	0.0315*	3.276 ±3.582	0.1730
	Yes	6.832 ±4.403		6.944 ±8.972	
FIGO staging[1]	Early stage (I+II)	2.833±2.863	0.5833	3.983 ±5.208	0.2873
	Late stage (III + IV)	3.989 ±4.094		6.396 ±7.041	
Grade[1]	Low grade (I+ II)	3.776±3.825	0.6771	6.758 ±7.811	0.6519
	High grade (III)	3.847±3.906		4.632 ±4.405	
Recurrence of the disease[1]	No	3.85±3.84	0.7256	5.762 ±5.466	0.3181
	Yes	3.373±3.809		5.498 ±8.072	
Distant metastasis [1]	No	3.01±2.91	0.5214	5.613 ±6.244	0.9947
	Yes	4.678 ±4.777		5.713 ±7.202	
CA-125	<35 (U/ml)	4.263±3.947	0.9646	7.17 ±7.271	0.8491
	>35 (U/ml)	4.317±4.456		6.252 ±5.93	
Response to chemotherapy [1]	Sensitive	4.1±3.806	0.4993	7.241±7.117	0.0457*
	Resistant	3.089±4.421		2.761±3.5	

BMI: Body Mass Index; SD: standard deviation; FIGO International Federation of Gynaecology and Obstetrics;
[1] p value performed with Mann-Whitney test; [2] p value performed with Kruskal-Wallis test * p<0.05

Discussion

EOC is a common gynecologic disease with an insidious onset that makes early detection difficult and is responsible for a high mortality rate (https://gco.iarc.fr/). Elucidating factors associated with higher risk and development and/or response to chemotherapy of EOC is essential to improve diagnosis and prognosis. miRNAs have been shown to influence numerous cellular processes such as proliferation, differentiation, and apoptosis. Their aberrant expression has been well documented in various diseases, including cancer [19]. Fortunately, miRNAs are stable and easily detected in plasma, so they have potential value as diagnostic biomarkers for multiple cancers, including EOC [20]. In this regard, the association of dysregulated plasma levels of miR-21, miR-200a, miR-200b, miR-200c, miR-205, and miR-125b, and the risk of EOC was documented in several populations [21-24]. In this study, we also confirmed the upregulation of circulating miR-21 and miR-125b in EOC patients compared to healthy controls. Located on chromosome [17], miR-21 is one of the earliest identified cancer-promoting "oncomiR" targeting numerous tumour suppressor genes associated with proliferation, apoptosis, and invasion [25]. Our result shows that the expression of miR-21 was twice as high in EOC patients compared to healthy subjects ($p<0.05$). This is consistent with Chinese [26,27], Egyptian [28], American [23], and Indian studies [29], which also documented increased levels of miR-21 in ovarian cancer patients. Furthermore, other studies have also demonstrated the overexpression of miR-21 in many malignancies such as breast cancer [30], head, and neck cancer [31,32], Hodgkin lymphoma [33], chronic myeloid leukemia [34], colon cancer [35], prostate cancer [36], brain tumour [37], cholangiocarcinoma [38], lung cancer [39], esophageal cancer [40] and pancreatic cancer [41] supporting the potential diagnostic and prognostic value of miR-21.

The increased levels of miR-21 can be explained by its close association with carcinogenesis, as it has been

shown to interfere with cell survival by regulating the cell cycle, apoptotic proteins, metalloproteinases, and others [42]. Thus, miR-21 is a potential key factor in tumour growth and the initiation, progression, invasion, and metastasis in various tumours, including EOC.

MiR-125b is ubiquitously expressed with the highest expression in the ovaries and brain (http://www.microrna.org/). miR-125b is dysregulated in a variety of tumours. Some studies have reported upregulation of miR-125b in some tumours such as colorectal cancer [43], gastric cancer [44], follicular carcinoma [45], and pancreatic cancer [46], suggesting an oncogenic potential of miR-125b. However, others have documented its downregulation in head and neck tumours [47], oral squamous cell carcinoma [48], osteosarcoma [49], bladder cancer [50,51], and thyroid cancer [45], highlighting its tumour-suppressive potential.

In this study, we demonstrated that plasma levels of miR-125b were upregulated in patients with EOC compared to healthy controls. Our results are consistent with an Indian study [52] and a Chinese study [53] which documented that circulating miR-125b levels were higher in women with EOC than in healthy controls.

However, Chinese and Italian studies performed on tissues [54-57] reported downregulation of miR-125b in EOC tissues compared with controls. This may be explained by the biological samples studied and by the different oncogenes and tumour suppressor genes targeted by miR-125b. The molecular mechanisms leading to the down or up-regulation of miR-125b in this type of cancer are not yet fully understood. To better validate our findings, we must study the expression of these miRNA in tissues from EOC patients and identify their target genes.

The present study found no significant difference in miR-21 and miR-125b levels when age, BMI, menarche, menopause, FIGO stage, disease recurrence, and distant metastases were considered. However, a large sample size and additional studies are needed to elucidate better the potential association between these clinicopathological features and dysregulated miRs.

In advanced EOC, the first line of chemotherapy consists of the combination of carbo/cisplatin and paclitaxel; unfortunately, 20% of patients do not respond to treatment 58. Our result indicates that decreased level of miR-125b is associated with chemoresistance. This is consistent with the findings of Chen and Sorrentino, who found downregulation of miR-125b in chemo resistant patients compared to those who responded to treatment [59,60]. This can be explained in part by the fact that a significant decrease in miR-125b expression levels leads to an increase in the expression levels of the anti-apoptotic factor BCL-2, which is a direct target of this miR. In other studies, miR-125b was upregulated in chemo resistant patients [60,61], suggesting that the upregulation of miR-125b leads to significant inhibition of bak-1, a pro-apoptotic regulator involved in a variety of cellular activities and also a direct target of miR-125b. Consequently, downregulation of bak-1 suppresses cisplatin-induced cytotoxicity and apoptosis and, thus, resistance to cisplatin [61].

Conclusion

The present study revealed significant upregulation of circulating miR-21 and miR-125b in EOC patients compared with healthy controls. Moreover, decreased miR-125b was detected in EOC patients who developed resistance to treatment, highlighting the potential role of miR-21 and miR-125b as future theranostic biomarkers in EOC.

Acknowledgements

We thank all blood donors and patients with EOC who volunteered to participate in the present study. We thank the staff of the Salah Azaiez Oncology Institute for their help in collecting blood samples

The study was supported in part by the Tunisian Ministry of Higher Education and Scientific research and the PRFD4-P1-ANPR project.

Conflict of interest

The authors declare no conflict of interest

Informed Consent

The study protocol has been approved by the Ethics Committee of SAI and that all participants have provided informed consent, and the approval code is ISA/2019/01

References

1. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. The Lancet. 2014; 384(9951): 1376-1388.
2. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. *Cancer Biology & Medicine*. 2017; 14(1): 9-32.

3. Gibson SJ, Fleming GF, Temkin SM, Chase DM. The Application and Outcome of Standard of Care Treatment in Elderly Women with Ovarian Cancer: A Literature Review over the Last 10 Years. *Frontiers in Oncology*. 2016; 6: 63.
4. Stordal B, Hamon M, McEneaney V, et al. Resistance to paclitaxel in a cisplatin-resistant ovarian cancer cell line is mediated by P-glycoprotein. *PLoS One*. 2012; 7(7): e40717.
5. Cooke SL, Brenton JD. Evolution of platinum resistance in high-grade serous ovarian cancer. *The Lancet Oncology*. 2011; 12(12): 1169-74.
6. Chen SN, Chang R, Lin LT, et al. MicroRNA in Ovarian Cancer: Biology, Pathogenesis, and Therapeutic Opportunities. *International Journal of Environmental Research and Public Health*. 2019; 16(9):1510.
7. Tucci P. The Role of microRNAs in Cancer: Functions, Biomarkers and Therapeutics. Cancers. 2022;14(4):872.
8. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*. 1993 75(5):843-854.
9. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Frontiers in Endocrinology*. 2018; 9:402.
10. Palmero EI, de Campos SG, Campos M, et al. Mechanisms and role of microRNA deregulation in cancer onset and progression. *Genetics and Molecular Biology*. 2011; 34(3):363-70.
11. Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death & Differentiation. 2010 ;17(2):215-220.
12. Creighton CJ, Fountain MD, Yu Z, et al. Molecular profiling uncovers a p53-associated role for microRNA-31 in inhibiting the proliferation of serous ovarian carcinomas and other cancers. *Cancer Research* 2010; 70(5):1906-15.
13. Banzhaf-Strathmann J, Edbauer D. Good guy or bad guy: the opposing roles of microRNA 125b in cancer. *Cell Communication and Signaling*. 2014; 12: 30.
14. Iorio MV, Visone R, Di Leva G, et al. MicroRNA signatures in human ovarian cancer. *Cancer Research*. 2007; 67(18):8699-707.
15. Nakamura K, Sawada K, Yoshimura A, Kinose Y, Nakatsuka E, Kimura T. Clinical relevance of circulating cell-free microRNAs in ovarian cancer. *Molecular Cancer*. 2016; 15(1): 48.
16. Nassar FJ, Talhouk R, Zgheib NK, et al. microRNA Expression in Ethnic Specific Early-Stage Breast Cancer: An Integration and Comparative Analysis. *Scientific Reports*. 2017; 7(1): 1689.
17. Nassar FJ, Chamandi G, Tfaily MA, Zgheib NK, Nasr R. Peripheral Blood-Based Biopsy for Breast Cancer Risk Prediction and Early Detection. *Frontiers in Medicine*. 2020; 7: 28
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001; 25:402-8 PubMed .
19. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *British Journal of Cancer*. 2007; 96 Suppl: R40-R44.
20. Brase JC, Wuttig D, Kuner R, Sültmann H. Serum microRNAs as non-invasive biomarkers for cancer. *Molecular Cancer*. 2010; 9: 306.
21. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer [published correction appears in Gynecol Oncol. 2010 Jan; 116(1): 153]. *Gynecologic Oncology*. 2008; 110(1): 13-21.
22. Ayaz L, Çayan F, Balci Ş, et al. Circulating microRNA expression profiles in ovarian cancer. *Journal of Obstetrics and Gynaecology*. 2014; 34(7): 620-24.
23. Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. Differentially expressed microRNAs are detected from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecologic Oncology* .2009; 112(1): 55-59.
24. Kan CW, Hahn MA, Gard GB, et al. Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. *BMC Cancer*. 2012; 12: 627.
25. Feng YH, Tsao CJ. Emerging role of microRNA-21 in cancer. Biomedical Reports. 2016; 5(4): 395-402.
26. Xu YZ, Xi QH, Ge WL, Zhang XQ. Identification of serum microRNA-21 as a biomarker for early detection and prognosis in human epithelial ovarian cancer. *Asian Pacific Journal of Cancer Prevntion*. 2013; 14(2): 1057-60.
27. Zheng H, Zhang L, Zhao Y, et al. Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer. *PLoS One*. 2013; 8(11): e77853.
28. Mahmoud EH, Fawzy A, A Elshimy RA. Serum MicroRNA-21 Negatively Relates to Expression of Programmed Cell Death-4 in Patients with Epithelial Ovarian Cancer. *Asian Pacific Journal of Cancer Prevention*. 2018; 19(1): 33-38.
29. Paliwal N, Vashist M, Chauhan M. Evaluation of

miR-22 and miR-21 as diagnostic biomarkers in patients with epithelial ovarian cancer. 3 Biotech. 2020; 10(3):142.
30. Savari B, Boozarpour S, Tahmasebi-Birgani M, Sabouri H, Hosseini SM. Overexpression of microRNA-21 in the Serum of Breast Cancer Patients. *MicroRNA*. 2020; 9(1): 58-63.
31. Wang Y, Zeng G, Jiang Y. The Emerging Roles of miR-125b in Cancers. *Cancer Management and Research*. 2020; 12: 1079-1088.
32. Mahmood N, Hanif M, Ahmed A, et al. Circulating miR-21 as a prognostic and predictive biomarker in oral squamous cell carcinoma. *Pakistan Journal of Medical Sciences*. 2019;35(5):1408-1412.
33. Jones K, Nourse JP, Keane C, Bhatnagar A, Gandhi MK. Plasma microRNA are disease response biomarkers in classical Hodgkin lymphoma. *Clinical Cancer Research*. 2014; 20(1): 253-64.
34. Navabi A, Akbari B, Abdalsamadi M, Naseri S. The role of microRNAs in the development, progression and drug resistance of chronic myeloid leukemia and their potential clinical significance. *Life Sciences*. 2022; 296:120437.
35. Nassar FJ, Msheik ZS, Itani MM, et al. Circulating miRNA as Biomarkers for Colorectal Cancer Diagnosis and Liver Metastasis. *Diagnostics*. 2021;11(2):341.
36. Ibrahim NH, Abdellateif MS, Kassem SH, Abd El Salam MA, El Gammal MM. Diagnostic significance of miR-21, miR-141, miR-18a and miR-221 as novel biomarkers in prostate cancer among Egyptian patients. *Andrologia*. 2019;51(10): e13384.
37. Parviz Hamidi M, Haddad G, Ostadrahimi S, et al. Circulating miR-26a and miR-21 biomarkers for glioblastoma multiform. *Biotechnology and Applied Biochemistry* .2019;66(2):261-265.
38. Correa-Gallego C, Maddalo D, Doussot A, et al. Circulating Plasma Levels of MicroRNA-21 and MicroRNA-221 Are Potential Diagnostic Markers for Primary Intrahepatic Cholangiocarcinoma. *PLoS One*. 2016;11(9): e0163699.
39. Jiang HG, Dai CH, Xu YP, et al. Four plasma miRNAs act as biomarkers for diagnosis and prognosis of non-small cell lung cancer. *Oncology Letters*. 2021; 22(5):792.
40. Zhang L, Dong B, Ren P, et al. Circulating plasma microRNAs in detecting esophageal squamous cell carcinoma. *Oncology Letters*. 2018; 16(3): 3303-3318.
41. Qu K, Zhang X, Lin T, et al. Circulating miRNA-21-5p as a diagnostic biomarker for pancreatic cancer: evidence from comprehensive miRNA expression profiling analysis and clinical validation. *Scientific Reports*. 2017; 7(1): 1692.
42. Krichevsky AM, Gabriely G. miR-21: a small multi-faceted RNA. *Journal of Cellular and Molecular Medicine*. 2009; 13(1): 39-53.
43. Nishida N, Yokobori T, Mimori K, et al. MicroRNA miR-125b is a prognostic marker in human colorectal cancer. *International Journal of Oncology*. 2011; 38(5): 1437-43.
44. Li X, Zhang Y, Zhang H, et al. miRNA-223 promotes gastric cancer invasion and metastasis by targeting tumor suppressor EPB41L3. *Molecular Cancer Research*. 2011; 9(7): 824-33.
45. Vriens MR, Weng J, Suh I, et al. MicroRNA expression profiling is a potential diagnostic tool for thyroid cancer. *Cancer*. 2012; 118(13): 3426-32.
46. Zhou X, Lu Z, Wang T, Huang Z, Zhu W, Miao Y. Plasma miRNAs in diagnosis and prognosis of pancreatic cancer: A miRNA expression analysis. *Gene*. 2018; 673:181-193.
47. Hou B, Ishinaga H, Midorikawa K, et al. Circulating microRNAs as novel prognosis biomarkers for head and neck squamous cell carcinoma. *Cancer Biology & Therapy*. 2015; 16(7):1042-1046.
48. Yap T, Koo K, Cheng L, et al. Predicting the Presence of Oral Squamous Cell Carcinoma Using Commonly Dysregulated MicroRNA in Oral Swirls. *Cancer Prevention Research*. 2018; 11(8):491-502.
49. Liu LH, Li H, Li JP, et al. miR-125b suppresses the proliferation and migration of osteosarcoma cells through down-regulation of STAT3. *Biochemical and Biophysical Research Communications*. 2011; 416(1-2): 31-8.
50. Wen Z, Huang G, Lai Y, et al. Diagnostic panel of serum miR-125b-5p, miR-182-5p, and miR-200c-3p as non-invasive biomarkers for urothelial bladder cancer [published online ahead of print, 2022 Jan 14]. *Clinical and Translational Oncology*. 2022; 10.1007/s12094-021-02741-3.
51. Lin T, Dong W, Huang J, et al. MicroRNA-143 as a tumor suppressor for bladder cancer. *The Journal of Urology*. 2009; 181(3): 1372-1380.
52. Zuberi M, Khan I, Mir R, Gandhi G, Ray PC, Saxena A. Utility of Serum miR-125b as a Diagnostic and Prognostic Indicator and Its Alliance with a Panel of Tumor Suppressor Genes in Epithelial Ovarian Cancer. *PLoS One*. 2016; 11(4): e0153902.
53. Zhu T, Gao W, Chen X, et al. A Pilot Study of Circulating MicroRNA-125b as a Diagnostic and Prognos-

tic Biomarker for Epithelial Ovarian Cancer. *International Journal of Gynecological Cancer*. 2017; 27(1): 3-10.
54. Luo S, Wang J, Ma Y, Yao Z, Pan H. PPARγ inhibits ovarian cancer cells proliferation through upregulation of miR-125b. *Biochemical and Biophysical Research Communications*. 2015; 462(2): 85-90.
55. Ying X, Wei K, Lin Z, et al. MicroRNA-125b Suppresses Ovarian Cancer Progression via Suppression of the Epithelial-Mesenchymal Transition Pathway by Targeting the SET Protein. *Cellular Physiology & Biochemistry*. 2016; 39(2): 501-10.
56. He J, Jing Y, Li W, et al. Roles and mechanism of miR-199a and miR-125b in tumor angiogenesis. *PLoS One*. 2013; 8(2): e56647.
57. Gadducci A, Sergiampietri C, Lanfredini N, Guiggi I. Micro-RNAs and ovarian cancer: the state of art and perspectives of clinical research. *Gynecological Endocrinology*. 2014; 30(4): 266-71.
58. Kyrgiou M, Salanti G, Pavlidis N, Paraskevaidis E, Ioannidis JP. Survival benefits with various chemotherapy regimens for ovarian cancer: a meta-analysis of multiple treatments. *Journal of the National Cancer Institute*. 2006; 98(22):1655-1663.
59. Chen Z, Guo X, Sun S, Lu C, Wang L. Serum miR-125b levels associated with epithelial ovarian cancer (EOC) development and treatment responses. *Bioengineered*. 2020; 11(1): 311-317.
60. Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. *Gynecologic Oncology*. 2008; 111(3): 478-486.
61. Kong F, Sun C, Wang Z, et al. miR-125b confers resistance of ovarian cancer cells to cisplatin by targeting pro-apoptotic Bcl-2 antagonist killer1. *Journal of Huazhong University of Science and Technology- Medical Science*. 2011; 31(4): 54.