Journal of Molecular and Genetic Medicine

Saeidi et al., J Mol Genet Med 2018, 12:3
DOI: 10.4172/1747-0862.1000358

Research Article Open Access

Evaluation of Circulating miRNA146a, miRNA155 and miRNA373 as Potential Biomarkers in Ovarian Cancer Detection

Saeidi N*, Saeidi G, Kheirandish S, Rashkuiyeh ZZR, Kalantar SM, Sheikhha MH and Firoozabadi NG

Shahid Sadoughi University of Medical Sciences and Health Services, Yazd Province, Iran

Abstract

MicroRNAs (miRNAs) are a special class of small non-coding RNAs that play a key role in gene regulations. In recent decades, many studies have suggested that miRNA deregulation is related to cancer development. Nevertheless, ovarian cancer (OC)-associated miRNA investigations are generally limited to tissue miRNA. In the present survey, we evaluated the specific profiles of serum-derived miRNAs of OC. The expression profiles of three miRNAs in the serum of the 31 OC and 28 healthy female subjects were examined by the Real-time-PCR technique. Our findings revealed that expression levels of the three examined miRNAs were significantly upregulated in OC subjects versus the control group ($p<0.05$). Indeed, miRNA146a, miRNA155, miRNA373 were overexpressed only in the OC group and these miRNAs seemed to be OC specific markers, which could be regulated by methylation and histone changes. In conclusion, the presented study proposed that the identification of these OC-specific miRNAs in serum might be considered as innovative non-invasive biomarkers.

Keywords: MicroRNA; Ovarian cancer; Real-time PCR

Introduction

Recent medical researchers have detected the new reliable biomarkers as diagnostic and prognostic markers for various disorders. In this regards, miRNAs as non-coding, small single strand RNAs, that controls the wide range of extracellular and intracellular activities could be considered as main diagnostic biomarkers. miRNA could be detected in tissues and body fluids such as urine and blood. Screening the miRNAs in body fluids are not only noninvasive but also economically feasible for diagnostic and prognostic aims. Overall, evaluation of the serum miRNAs used for assessment of the pre and post irradiation therapy outcomes. In addition, miRNAs could be applied as novel biomarkers in cancer diagnosis and treatments such as breast cancer hepatocellular carcinoma and colorectal cancer.

Ovarian cancer (OC) is considered as a prevalent cancer related to a high rate of mortality in women [1,2]. Lack of screening tests for ovarian cancer leads to late cancer diagnosis in advanced stages, resulting in a high morbidity rate. In other words, recognizing this illness is extremely difficult, and is, consequently, associated with decreased patient survival [1,3,4].

Of late, it has become important to pay attention to evaluating specific biomarkers that may help in early diagnosis and, thus, decrease cancer-related mortality [5]. Assessment of miRNA profiles had been considered as one of the perceptible tool to examine the miRNA expression in various types of human cancers. MiRNA evaluation presented an experimental evidence, showing the function of these small noncoding RNAs in tumorigenesis [6]. MiRNAs are single-strand non-coding RNAs that contain 19–25 nucleotides. MiRNAs primarily produce as long miRNAs precursor via RNA II polymerase-mediated transcription and are then cleaved and transported into the cytoplasm [7,8]. Major surveys have indicated that miRNA dysregulation is present in various types of human disorders such as ovarian cancer [7,9,10]. Among different miRNA types, the modified expression levels of miRNA-146a have been associated with cancer progression. In addition, this miRNA has negative regulative function on inflammatory precursors, which is related to an innate immune response [11]. MiRNA-155 is another miRNA which is overexpressed in numerous cancers. It seems that miRNA-155 acts as an oncomiRNA in neoplasia such as breast cancer by repressing the cytokine signaling 1 gene. MiRNA-373 is a human embryonic stem cell (ESC)-specific miRNA with potentially novel oncogenic effects. It is able to induce proliferation and carcinogenesis of human cell anchorage to oncogenic agents including RAS and wild-type p53 [12-14]. MiRNA-373 was studied in several tumors, including gastric cancer, esophageal cancer, colon cancer, prostate cancer, liver cancer, pancreatic cancer, and lung cancer. MiRNA-373 acts as an oncogene or a tumor suppressor in accordance with its regulation in neoplasms compared to non-cancer specimens [14]. Various types of tumors have a dissimilar miRNA expression range. Indeed, extensive studies have been performed to evaluate the normal ovarian cell and ovarian neoplasmic tissues in the miRNA expression status [15,16]. According to an earlier survey on ovarian cancer, some miRNAs such as miRNA-141, miRNA-200a, miRNA-200b, and miRNA-200c were overexpressed, some miRNAs down-regulated (for instance, miRNA-125b-1, miRNA-140, miRNA-145, and miRNA-199a had the highest multiples of down-regulation) [15]. According to the author's knowledge, we could not find any study that evaluated the circulating miRNA146a, miRNA155, and miRNA373 profiles in the pathogenesis of OC, and discussed increasing efforts to apply them as new diagnostic tools for effective future OC diagnosis.

Materials and Methods

The study protocols were approved by the Ethical Committee of Yazd University of Medical Sciences, Yazd, Iran. Each participant signed her written consent and ethical statements. Thirty-one OC patients referred to the Yazd infertility clinic, Iran, who underwent abdominal surgery between 2002 and 2014, were enrolled in this case-

***Corresponding author:** Nasim Saeidi, Shahid Sadoughi University of Medical Sciences and Health Services Yazd Province, Iran, Tel: +98 (35) 3725 8410; E-mail: saeidi.nsm@gmail.com

Received July 06, 2018; **Accepted** August 05, 2018; **Published** August 10, 2018

Citation: Saeidi N, Saeidi G, Kheirandish S, Rashkuiyeh ZZR, Kalantar SM, et al. (2018) Evaluation of Circulating miRNA146a, miRNA155 and miRNA373 as Potential Biomarkers in Ovarian Cancer Detection. J Mol Genet Med 12: 358 doi:10.4172/1747-0862.1000358

controlled study to evaluate the expression levels of miRNA146a, miRNA155, miRNA373 compared to the healthy control group. A total histopathological examination was performed in all cases, which assured accurate tumor staging. OC patients' clinical data was obtained from medical records and follow-up of all subjects done in the Yazd infertility clinic, Iran. The healthy control group consisted of 28 female subjects, who had been referred to the Yazd blood transfusion center, Yazd, Iran, without any neoplasmic diseases, participated in this study. In following, 5 ml of baseline blood samples were drawn in EDTA before the administration of chemotherapy or radiotherapy. The blood specimens were centrifuged (12000 g for 15 minutes at 4°C) within 1 h of venipuncture and stored at 70°C until use. The number of samples in the control group was similar to that in the OC group.

MicroRNA analysis

RNA was isolated from serum using the manual Trizol reagent. In this method, 750 µl of TRIZOL Reagent (Reagent AMBION) was poured into a fresh tube and 750-µl of serum added to the TRIZOL reagent. An insufficient amount of TRIZOL Reagent can lead to DNA contamination of the extracted RNA. The vortex mixer was then used for 15 s to permit the complete dissociation of nucleoprotein complexes. The homogenized mixture was incubated at room temperature (RT) for 10 min. and µl of 200 chloroform was added and shacked. The samples were centrifuged at 12,000 × g for 20 min. at 4°C. Following centrifugation, the mixture separated into three phases, of which the upper aqueous phase was transferred to a fresh tube (RNA present entirely in the aqueous phase). RNA precipitated by mixing with isopropyl alcohol (Merck, Germany) (the precipitated RNA, formed a gel-like pellet). Then the mixture was incubated in RT for 15 min. and centrifuged (12000 × g, at 4°C, 15 min.). Next, the RNA pellet was washed with 75% ethanol. Then the specimens were mixed by vortexing and centrifuged (12000 × g, at 4°C, 8 min). All leftover ethanol was removed. RNA pellets were air-dried and dissolved in deionized water.

Then, ng 30 of total RNA was reverse-transcribed using the RNA cDNA synthesis kit as prescribed in the protocol guidelines (EURX, South Korea,) using a VERITI device (Applied biosystem-2209294500). The primer sequences of miRNA-146-a, miRNA-155, and miRNA-373 for cDNA synthesis (Table 1).

In the next step, the cDNA samples were mixed with Real-time SYBRgreen2X Master Mix (Ampliqon, Denmark), then forward and conventional primers added, and the mixture applied to the Thermocycler System Real-time PCR device (QIAGENE, Germany). The optimized Real-time PCR cycling conditions (Table 2).

All samples were run in triplicate. The comparative threshold (Ct) was utilized to detect the relative expression level of each miRNA. MiRNA103 levels were used to normalize expression. The Ct analysis was performed by the 2– Ct method [16,17].

Statistical analysis

All the tests were performed in triplicate. The data represented as mean ± standard error. The statistical differences among groups were studied by the Student's t-test. $p<0.05$ indicated a significant difference.

Results

Thirty-one patients with epithelial OC and 28 healthy females participated in this study. The demographic characteristics of patients and controls presented in Tables 3 and 4.

After adjusting the subjects' characteristics, the serum samples were collected and analyzed. According to our results, the median age of the patients was 35 to 60 years. The demographic characteristics of patients in the two groups did not have any significant difference ($p>0.05$).

In this survey, three unique miRNAs including miRNA146a, miRNA155, and miRNA373 were profiled. Indeed, we could measure different miRNAs in all samples (OC and control groups). Utilizing serum isolated miRNAs, we identified the circulating miRNA expression profiles of pre-surgical samples from 31 women with confirmed serious epithelial ovarian cancer, compared to healthy women. The miRNA expression profiling by total RNA extraction from serum samples of controls and patients with advanced-stage OC (Stage III) was successfully identified. In order to detect a diagnostic model for the recognition of OC, we utilized different criteria. We detected miRNA levels that showed statistically significant differences in OC patients compared to healthy subjects.

All other three examined miRNAs satisfied our criteria of being expressed in majority samples of each group and demonstrating an increased fold change. miRNA146a overexpressed in plasma in cancer patients compared to controls ($p<0.05$). Serum samples from healthy women had significantly lower levels of miRNA146a (Figure 1).

Furthermore, miRNA155 expression between pre-surgical samples

hsa-miR-146a-5p	Stem loop	5′GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAACCCA3′
	Forward	5′CACGCATGAGAACTGAATTCCA3′
hsa-miR-373-5p	Stem loop	5′GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGGAAAG3′
	Forward	5′ CACGCAACTCAAAATGGGGGCG3′
hsa-miR-155-5p	Stem loop	5′GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACCCCT3′
	Forward	5′ CACGCATTAATGCTAATCGTGAT3′
Conventional	-	5′-CCA GTG CAG GGT CCG AGG'TA-3
hsa-miR-103a-3p	Stem loop	5′GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCATAG3′
	Forward	5′ CACGCAAGCAGCATTGTACAGGG3′

Table 1: Primers sequences of miRNA-146-a, miRNA-155 and miRNA-373 for real cDNA synthesis.

Variables	Temperature	Time	Repeated Cycles of Temperatures
Hold 1	95°C	15 min	1
Cycling 1	95°C	30 sec	40
	62°C	30 sec	
	72°C	30 sec	

Table 2: Optimized real-time PCR cycling conditions.

Alcohol Drinking	Smoking	The Genetic History of Ovarian Cancer in First Degree Relatives	Miscarriage History	Age at First Pregnancy	Breastfeeding to Her Infant(S)	The Duration of Breastfeeding (Months)	Number of Children	History of Cancer	Menopause	Age of Menarche	Age	No.
No	No	No	No	16	Yes	24	4	No	No	12	45	1
No	No	No	No	15	Yes	18	5	No	No	11	55	2
No	No	No	No	15	Yes	18	6	No	No	10	50	3
No	No	No	No	17	Yes	18	3	No	No	13	35	4
No	No	No	5	16	Yes	18	4	No	No	14	48	5
No	No	No	No	16	Yes	24	6	No	No	13	54	6
No	No	No	No	18	Yes	18	2	No	No	12	39	7
No	No	No	No	17	Yes	24	3	No	No	13	40	8
No	No	No	1	14	Yes	24	5	No	55	11	59	9
No	No	No	No	17	Yes	24	3	No	No	13	46	10
No	No	No	No	16	Yes	18	6	No	No	12	50	11
No	No	No	No	15	Yes	24	6	No	50	14	60	12
No	No	No	No	16	Yes	24	4	No	No	12	52	13
No	No	No	No	16	Yes	18	7	No	50	14	54	14
No	No	No	No	16	Yes	18	5	No	53	13	58	15
No	No	No	No	17	Yes	18	4	No	No	11	37	16
No	No	No	No	17	Yes	24	5	No	56	13	56	17
No	No	Sister	No	16	Yes	24	6	No	No	13	54	18
No	No	No	No	15	Yes	24	8	No	56	13	61	19
No	No	No	No	14	Yes	30	4	No	No	12	50	20
No	No	No	No	16	Yes	24	5	No	No	14	47	21
No	No	No	No	17	Yes	24	6	No	No	14	53	22
No	No	No	1	17	Yes	18	5	No	No	13	49	23
No	No	No	No	16	Yes	18	8	No	No	13	55	24
No	No	Sister	No	16	Yes	18	4	No	55	15	62	25
No	No	No	No	16	Yes	18	5	No	55	13	64	26
No	No	No	No	15	Yes	24	4	No	54	12	57	27
No	No	No	No	17	Yes	24	3	No	No	13	43	28
No	No	No	No	17	Yes	18	2	No	No	13	42	29
No	No	Sister	No	17	Yes	24	2	No	No	12	35	30

Table 3: The demographic characteristics of ovarian cancer patients.

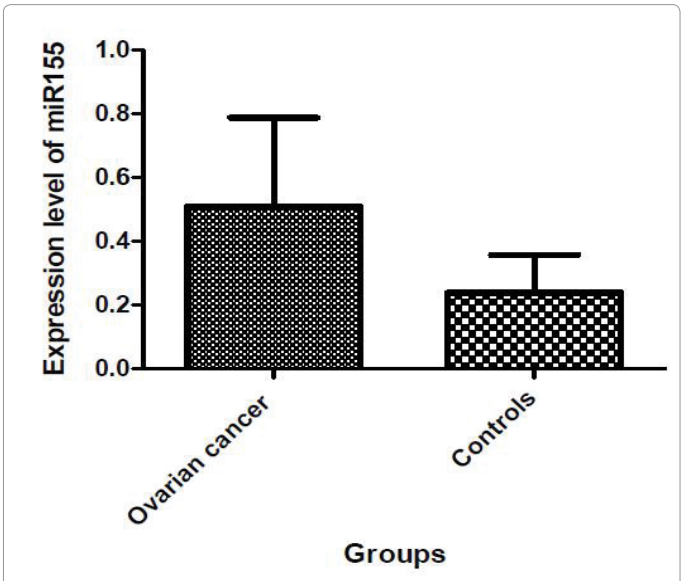

Figure 1: Levels of miRNA-146a in ovarian cancer (OC) and control groups. Expression levels detected in serum samples from OC patients (n=31) or controls (n=28). Data expressed as mean fold change ± SEM.

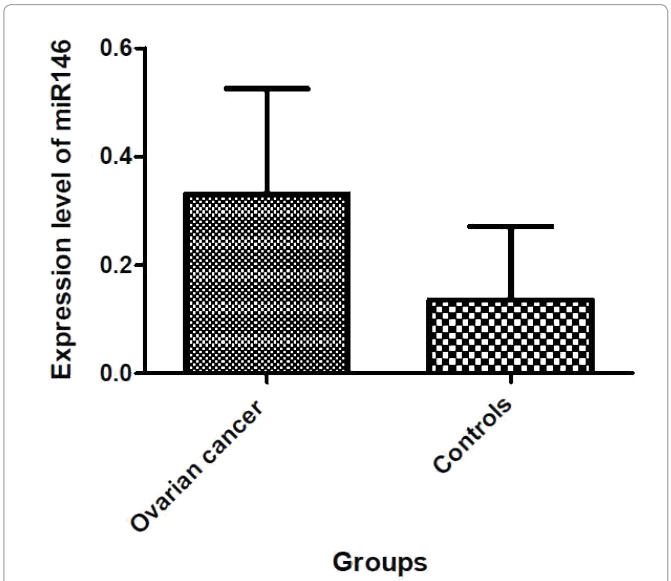

Figure 2: Level of miRNA-155 in ovarian cancer (OC) and control groups. Expression levels detected in serum samples from OC patients (n=31) or controls (n=28).data expressed as mean fold change ± SEM.

Alcohol drinking	Smoking	The Genetic History of Ovarian Cancer in First Degree Relatives	Miscarriage History	Age at first pregnancy	Breastfeeding to her infant(s)	The Duration of Breastfeeding (Months)	Number of Children	History of Cancer	Age of Menopause	Age of Menarche	Age	No.
No	No	No	No	17	Yes	24	2	No	No	12	35	1
No	No	No	No	18	Yes	18	3	No	No	13	35	2
No	No	No	1	19	Yes	24	2	No	No	13	37	3
No	No	No	No	18	Yes	24	2	No	No	13	39	4
No	No	No	No	16	Yes	18	4	No	55	13	62	5
No	No	No	2	16	Yes	24	5	No	56	15	63	6
No	No	No	No	15	Yes	24	8	No	55	13	64	7
No	No	No	1	14	Yes	18	6	No	54	12	60	8
No	No	No	No	15	Yes	18	7	No	55	11	55	9
No	No	No	No	14	Yes	18	5	No	53	13	58	10
No	No	No	1	16	Yes	18	4	No	No	12	45	11
No	No	No	No	15	Yes	24	5	No	55	14	56	12
No	No	No	No	17	Yes	24	6	No	No	13	52	13
No	No	No	No	16	Yes	24	4	No	No	13	47	14
No	No	No	No	16	Yes	18	5	No	No	12	43	15
No	No	No	No	17	Yes	24	6	No	54	13	56	16
No	No	No	No	16	Yes	24	6	No	No	14	50	17
No	No	No	No	17	Yes	24	8	No	No	13	51	18
No	No	No	No	17	Yes	18	7	No	55	10	58	19
No	No	No	No	17	Yes	24	5	No	No	12	47	20
No	No	No	No	15	Yes	18	4	No	No	13	55	21
No	No	No	No	16	Yes	24	6	No	55	13	56	22
No	No	No	1	16	Yes	24	7	No	No	12	53	23
No	No	No	No	17	Yes	18	3	No	No	15	49	24
No	No	No	No	17	Yes	24	5	No	No	14	52	25
No	No	No	No	17	Yes	24	5	No	No	12	50	26
No	No	No	No	16	Yes	18	6	No	54	11	57	27
No	No	No	No	16	Yes	18	4	No	No	13	53	28
No	No	No	No	18	Yes	18	5	No	No	13	40	29
No	No	No	No	19	Yes	18	6	No	No	11	45	30

Table 4: The demographic characteristics of healthy control women.

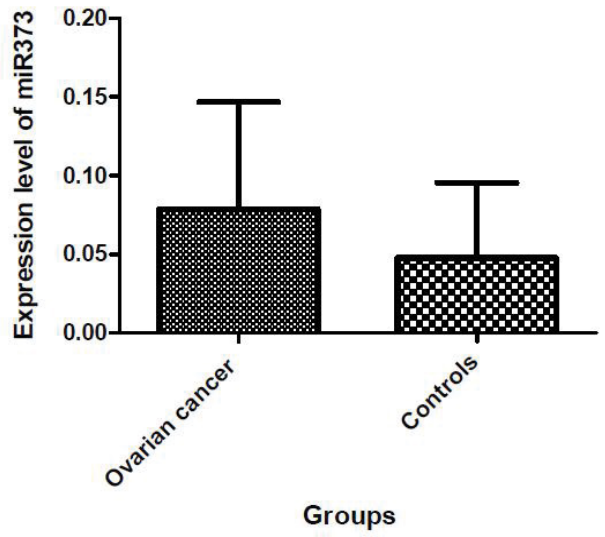

Figure 3: Level of miRNA373I in ovarian cancer (OC) and control groups. Expression levels detected in serum samples from OC patients (n=31) or controls (n=28).data expressed as mean fold change ± SEM.

and normal controls showed a differential expression pattern. Indeed, we found significantly elevated levels of miRNA155 in OC patients compared to control subjects (p<0.05, Figure 2).

In further analysis, we compared the pre-surgical serum miRNA373I profiles of OC-affected women and healthy women. Significant differences in miRNA373 expression levels were found in women having OC compared to controls (p<0.05; Figure 3). In the comparison of healthy women with cancerous serum samples, there were three miRNAs, (miRNA146a, miRNA155, miRNA373) which were differentially expressed. Indeed, all studied miRNAs were elevated only in cancer samples (p<0.05); so it seems that they could be considered as valuable markers in the differentiation between control and cancer samples.

Discussion

Recent evidence suggests that miRNAs have important roles in several important cellular events such as development, differentiation, cell cycle, and apoptosis [18]. It had been reported that miRNA expression patterns might be valuable markers in the diagnosis of various cancer types and patients' outcome. The evaluation of miRNAs in normal tissues compared to cancerous organs by various procedures has shown enormous deregulated miRNA genes [18,19].

In order to probe the several unanswered questions about the usefulness of circulating miRNAs as biomarkers in OC and the usage of circulating miRNAs as cancer biomarkers with highly accurate and precise evaluation, (20), we examined serum samples of women diagnosed with OC and compared with healthy controls. The expression

levels of all three studied miRNAs in OC patients had statistically significant differences than controls. According to these data, it seems that all three examined miRNAs were validated, as these results were able to predict the OC-specific diagnosis and patients' outcome.

Changes in the miRNA-146a expression have been shown in the development of various cancer types. Indeed, it seems that miRNA-146a expression is associated with the transcription factor NF-kappaB, which is involved in inflammation and cancer [11].

There are little data on the miRNA-146a expression level in ovarian tumors [20,21]. Earlier data revealed that miRNA-146a expression in primary tumor cells was significantly elevated compared to normal ovarian tissues [21]. In addition, Wyman et al. reported the lower expression levels of miRNA-146a in ovarian tumor compared to normal ovarian cells [22]. These inconsistencies in our results may be due to various histological types of cancer that were considered. Besides, the studied specimens were cultured human ovarian surface epithelial cells [21].

In an earlier study, the circulating miRNA-146a level was significantly elevated in plasma specimens of breast cancer subjects compared to those of controls [23]. In addition, a statistically significant upregulation of miRNA-146a was found in the serum of patients with uveal melanoma [24], which were in accordance with our results. Last reports suggested the higher expression of miRNA-146a in human nasopharyngeal carcinoma than in chronic nasopharyngitis [25] and a potential role of variant alleles of miR-146a alleles, which are related to the higher risk of squamous cell carcinoma of the head and neck [26]. According to a recent study, miRNA-146a overexpression reduced the NF-κB levels and the NF-κB - dependent extrinsic apoptotic pathway (TNF, FADD and TRADD) [27].

According to a previous report, miRNA-155 upregulates Mxd1/Mad1 of basic a helix-loop-helix leucine zipper transcription factor network and is associated with cellular proliferation, and apoptosis exerted by BCL6 regulation. So, miRNA-155 results in cell death resistance [28]. Resnick et al. evaluated 21 serum miRNAs among epithelial ovarian cancer patients and healthy controls. They reported that miRNA-155 were significantly under expressed [29]. This difference with our findings may be related to various diagnosed cancer stages between the two studied populations.

Another study indicated that in OC-associated fibroblasts, miRNA-155 upregulated compared to normal fibroblasts [30]. An earlier study had revealed that the miRNA-155 downregulated in ovarian cancer-initiating cell (OCC), which is associated with claudin-1(CL) overexpression and the decrease of the OCC invasion. Indeed, miRNA-155 targets CLDN1 mRNA on the 3' UTR. CL expression levels reduced in miRNA-155-OCC. They indicated that miRNA-155 suppressed the proliferative and invasive OCC capability [31].

Considering metastasis as a main reason of cancer-related mortality in OC patients, miRNA-373 has been shown to act as an essential factor in tumorigenesis and metastasis. A recent report indicated that miRNA-373 expression decreased in human epithelial ovarian cancer (EOC) and Rab22a might be considered as a direct target of miRNA-373 [32]. MiRNA373 is known as a probable oncogene, which is related to tumorigenesis of human cells harboring the oncogenic RAS and p53 [14]. New evidence shows that downregulation of miRNA-373 decreased the growth of AGS cells and apoptosis induction. Indeed, overexpression of miRNA-373 in the HGC-27 cells is related to cell growth [33].

Conclusion

According to the author's knowledge, we have not found any survey on the serum expression levels of miRNA-373 and our study is the first such investigation. It is because of this fact that cancers are essential issues in modern medicine [34]. One of the main targets of cancer therapy being the prediction of patient response to treatments [35]. Therefore, more attempts are necessary to develop innovative strategies that can lead to higher treatment efficacy [36], which is dependent on the recognition of specific markers in cancer diagnosis. In this survey, we endorsed that the extraction of RNA and identification of miRNAs from the serum of OC patients seems to be a viable method. We suggest the using a Real-time PCR, to evaluate miRNAs as a diagnostic test for OC patients. Additionally, we offer that miRNAs may be potentially considered as an analytical method in OC. Based on our results, we suggested a profile that was subsequently examined on a set of 31 patients and 28 healthy controls. Out of the three miRNAs of interest that we selected, all miRNAs were proposed to detect OC [37-41].

References

1. Razi S, Ghoncheh M, Mohammadian-Hafshejani A, Aziznejhad H, Mohammadian M, et al. (2016) The incidence and mortality of ovarian cancer and their relationship with the Human Development Index in Asia. Ecancermedicalscience 10: 628.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, et al. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. Int J Cancer 127: 2893–2917.
3. Bray F, Jemal A, Grey N, Ferlay J, Forman D (2012) Global cancer transitions according to the Human Development Index (2008–2030): A population-based study. Lancet Oncol 13: 790–801.
4. Keng SL, Wahab SBA, Chiu LB, Yusuf A (2015) Awarenessof ovarian cancer risk factors among women in Malaysia: A preliminary study. Asian Pac J Cancer Prev 16: 537–540.
5. Katz B, Trope CG, Reich R, Davidson B (2015) MicroRNAs in ovarian cancer. 46: 1245-1256.
6. Iorio MV, Croce CM (2013) MicroRNA profiling in ovarian cancer. Methods Mol Biol 1049: 187-197.
7. Nakamura K, Sawada K, Yoshimura A, Kinose Y, Nakatsuka E, et al. (2016) Clinical relevance of circulating cell-free microRNAs in ovarian cancer. Mol Cancer 15: 48.
8. Kim VN (2005) MicroRNA biogenesis: Coordinated cropping and dicing. Nat Rev Mol Cell Biol 6: 376–385.
9. Kinose Y, Sawada K, Nakamura K, Kimura T (2014) The role of microRNAs in ovarian cancer. Biomed Res Int 2014: 249-393.
10. Perera RJ, Ray A (2007) MicroRNAs in the search for understanding human diseases. BioDrugs 21: 97-104.
11. Williams AE, Perry MM, Moschos SA, Larner-Svensson HM, Lindsay MA (2008) Role of miRNA-146a in the regulation of the innate immune response and cancer. Biochem Soc Trans 36: 1211-1215.
12. Jiang S, Zhang HW, Lu MH, He XH, Li Y, et al. (2010) MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 70: 3119-3127.
13. Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, et al. (2006) A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Cell 124: 1169–1181.
14. Wei F, Cao C, Xu X, Wang J (2015) Diverse functions of miR-373 in cancer. J Transl Med 13: 162.
15. Wang ZH, Xu CJ (2015) Research progress of microRNA in early detection of ovarian Cancer. Chinese Med J 128: 3363-3370.
16. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, et al. (2007) MicroRNA signatures in human ovarian cancer. Cancer Res 67: 8699–8707.
17. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data

using real-time quantitative PCR−Deltaand theDelta2(C(T)) method. Methods 25: 402–408.

18. Dahiya N, Morin PJ (2010) MicroRNAs in ovarian carcinomas. Endocrine-related cancer. 17: F77-F89.
19. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, et al. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 101: 2999–3004.
20. Shapira I, Oswald M, Lovecchio J (2014) Circulating biomarkers for detection of ovarian cancer and predicting cancer outcomes. British J Cancer 110: 976-983.
21. Wilczyński M, Zytko E, Szymańska B, Dzieniecka M, Nowak M, et al. (2017) Expression of miR -146a in patients with ovarian cancer and its clinical significance. Oncol Lett 14: 3207-3214.
22. Wyman SK, Parkin RK, Mitchell PS, Fritz BR, O'Briant K, et al. (2009) Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA cDNA libraries. PLoS One 4: e5311.
23. Kumar S, Keerthana R, Pazhanimuthu A, Perumal P (2013) Overexpression of circulating miRNA-21 and miRNA-146a in plasma samples of breast cancer patients. Indian J Biochem Biophys 50: 210-214.
24. Russo A, Caltabiano R, Longo A (2016) Increased levels of miRNA-146a in serum and histologic samples of patients with uveal melanoma. Frontiers Pharmacol 7:424.
25. Zhao Y, Chen X, Jing M, Du H, Zeng Y (2012) Expression of miRNA-146a in nasopharyngeal carcinoma is upregulated by Epstein-Barr virus latent membrane protein 1. Oncol Rep 28: 1237-1243.
26. Zhang S, Hu F, Liang H, Liu Y, Yang J, et al. (2017) Association between a miRNA-146a polymorphism and susceptibility to head and neck squamous cell carcinoma in Chinese patients: A meta-analysis of 8 case–control studies. PLoS ONE 12: e0186609.
27. Sandhu R, Rein J, D'Arcy M, Herschkowitz JI, Hoadley KA, et al. (2014) Overexpression of miR-146a in basal-like breast cancer cells confers enhanced tumorigenic potential in association with altered p53 status. Carcinogenesis 35: 2567-2575.
28. Higgs G, Slack F (2013) The multiple roles of microRNA-155 in oncogenesis. J Clin Bioinformatics 3: 17.
29. Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, et al. (2009) The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 112: 55-59.
30. Mitra AK, Zillhardt M, Hua Y (2012) MicroRNAs reprogram normal fibroblasts into cancer associated fibroblasts in ovarian cancer. Cancer Disc 2: 1100-1108.
31. Qin W, Ren Q, Liu T, Huang Y, Wang J (2013) MicroRNA-155 is a novel suppressor of ovarian cancer-initiating cells that targets CLDN1. FEBS Lett 587: 1434-1439.
32. Zhang Y, Zhao FJ, Chen LL (2014) MiR-373 targeting of the Rab22a oncogene suppresses tumor invasion and metastasis in ovarian cancer. Oncotarget 5: 12291-12303.
33. Zhang X, LiX, Tan Z (2013) MicroRNA-373 is upregulated and targets TNFAIP1 in human gastric cancer, contributing to tumorigenesis. Oncology Letters 6:1427-1434.
34. Mohamadi N, Kazemi SM, Mohammadian M (2017) Toxicity of cisplatin-loaded poly butyl cyanoacrylate nanoparticles in a brain cancer cell line: Anionic polymerization results. Asian Pacific J Cancer Prevent 18: 629-632.
35. Mohammadian M, Zeynali S, Azarbaijani AF, Ansari KMH, Kheradmand F (2017) Cytotoxic effects of the newly-developed chemotherapeutic agents 17-AAG in combination with oxaliplatin and capecitabine in colorectal cancer cell lines. Res Pharmaceu Sci 12: 517-525.
36. Sajjadiyan SZ, Ghadernejad H, Milani AT, Mohammadian M, Abdolahpour S, et al. (2016) Preparation of silibinin loaded pegylatedniosomal nanoparticles and investigation of its effect on MCF-10A human breastcancer cell line. Der Pharmacia Lettre 8: 70-75.
37. Tomasskałubiń B, Cha-Fendler J, Chowdhury D, Fendler W (2018) Potential of serum microRNAs as biomarkers of radiation injury and tools for individualization of radiotherapy. Transl Res 1: 1-2.
38. Han Q, Liu D, Convertino M, Wang Z, Jiang C, et al. (2018) miRNA-711 binds and activates TRPA1 extracellularly to evoke acute and chronic pruritus. Neuron 1: 1-2.
39. Guo J, Liu C, Wang W, Liu Y, He H, et al. (2018) Identification of serum miR-1915-3p and miR-455-3p as biomarkers for breast cancer. PloS one 13: e0200716.
40. An Y, Gao S, Zhao WC, Qiu BA, Xia NX, et al. (2018) Novel serum microRNAs panel on the diagnostic and prognostic implications of hepatocellular carcinoma. World J Gastroenterol 24: 2596-2604.
41. Nikolaou S, Qiu S, Fiorentino F, Rasheed S, Tekkis P, et al. (2018) Systematic review of blood diagnostic markers in colorectal cancer. Tech Coloproctology 22: 481-498.