MOSUL JOURNAL OF NURSING
Online ISSN: 2663-0311 - Print ISSN: 2311-8784
Website: https://mjn.mosuljournals.com

RESEARCH ARTICLE

EXPRESSION OF MIR-571 AND MIR-20A IN BREAST CANCER PATIENTS AS DIAGNOSTIC BIOMARKERS

Omed Khalid Salih [1], Dlnya Asad mohamad [2] *

1. *College of science, Biology department, University of sulaimani, Sulaimani, Iraq.*
2. *Molecular Biology, College of Science, Biology Department, University of Sulaimani, Sulaimani, Iraq.*

Corresponding author: Omed Khalid Salih
Email: omedkhalid11@gmail.com

ORCID

ABSTRACT
Micro RNAs (miRNAs) are small nucleic acids and they are non-coding type of RNA, which are composed of 19-25 nucleotides and work as significant post-transcriptional gene controls of multiple biological roles. Commonly, miRNAs negatively control gene expression by adhering to their particular messenger RNAs in which they generally bind to the 3'-UTR (UN translated region) of their target mRNAs and repress protein production by destabilizing the mRNA and translational silencing.

(mRNAs), for both mRNA degeneration or translational restraint, depending on the level of their complementation with target its mRNA chains. Unusual expression of those miRNAs was found to be associated etiologically with several diseases such as breast carcinoma. Diverse cellular pathways involved in breast cancer is developing such as cell proliferation, apoptosis,metastasis, chemoresistance, and cancer recurrence which are controlled by the oncogenic miRNA (oncomiR) or suppressor miRNA for tumor (tsmiR). In the present study, the representation levels of miR-571 and miR-20a, the most two studied miRNAs in breast cancer, were estimated in 40 breast cancer patients at Hiwa Hospital, Sulaimani city. Real-Time-Reverse Transcription-PCR (RT-PCR) was applied to evaluate the expression of miR-571 and miR-20a. The clinical data including breast cancer with different grades, a cancer patient with chemotherapy state (pre-chemotherapy), and (post-chemotherapy) conditions.

Results exhibited that both miR-571 and miR-20a expression is correlated with disease stage among patients taking chemotherapy. According to the results, chemotherapy increased the expression of miR-571, but reduced the expression of miR-20a in patients with breast cancer. We also found that chemotherapy had more effect on miR-571 expression in comparison with miR-20a. Furthermore, the effect of chemotherapy was higher in the early stages of breast cancer in comparison to other disease stages. In the coclusionusing both miR-571 and miR-20a as important biomarkers for detecting the the beneficial effects of chemotherapy on breast cancer.

Keywords: Breast cancer, miRNA, Detection, Chemotherapy, Grade, Bio marker.

Received: 15 September 2021, Accepted: 25 December 2021, Available online: 20 January 2022

INTRODUCTION

Breast cancer is known as the most commonly diagnosed malignancy that attacks women and is the main reason for mortality in women experiencing cancers. The risk of extending breast carcinoma for women rises within the general population (A. Bahrami et al., 2018). This is described by three morphological ranks and up to four distinct molecular subtypes (A. J. Murray and D. M. Davies, 2013).According to the St. Gallen (Jackisch et al., 2015: Aleskandarany et al., 2018). Breast carcinoma was clinically characterized under four main subtypes: triple-negative, human epidermal growth factor receptor 2 HER2-positive, luminal A and luminal B. This has been suggested that various circumstances, including hormonal contraceptives, estrogen coupled with progesterone products, alcohol, obesity, and hormone replacement treatment, can enhance the chance of breast cancer among women.

In opposition, some factors such as high parity, breastfeeding, young age at first childbirth, early menopause, and late menarche are known to decrease breast cancer risk. In developed countries, the hazard of revealing breast cancer for women is about 9 to 11% and, Genetic variation is the main reason for breast cancer clustering in families, whereas environment and shared lifestyle have a low impact (A. Bahrami et al., 2018). Amongst the long listing of risk factors connected by breast cancer, the important function of miRNAs, which are essentially little molecules accountable for regulation, has been recorded(G. Curigliano et al.,2017).Better biological understanding of breast cancer and identifying new biomarkers are necessary for the immediate investigation and for more reliable disease lamination and directors options. In modern times, display of miRNA has frequently identified as an essential control of both healthy and cancer cells biology (He L and Hannon, 2004: S. Kurozumi et al., 2017).

miRNAs are short non-coding RNA (ncRNA) particles ranging from (18- 22) nucleotides in length. It can alter gene representation negatively through targeting mRNAs and enhance both translation repression and RNA degeneration (He L and Hannon, 2004: S. Kurozumi et al., 2017). Commonly, conserved miRNAs have essential roles on different processes, such as cell reproduction, apoptosis, and metabolism (E. C. Lai, 2003: Katarina Cuk et al., 2013 and K. H. Lee et al 2009). Largest miRNAs family performs an essential function in carcinomas as oncogenes or tumors suppressor genes (Y. Takahashi et al, .2009).Some investigations have shown that unusual appearance of miRNAs might be involved in tumor production (P. S. Chen et al.,2017). Expression of miR-571 and miR-20a has been widely studied in numerous cancers such as breast cancer(L. Feng,.2012: Y. Wang et al,.2016 : P. Sharma et al .,2013: C. Zhou et al.,2014 and J. Cuiet al.,2015). More data has shown that aberrant expression of miR-571 and miR-20a may be connected to early detection [9]. Furthermore, miRNAs can be reported in body liquid, and the representation of miRNAs found in peripheral blood and it can be utilized as a biological label for the differential analysis and prediction of breast cancer (Li XF et al, .2018).

Considering the importance of miRNAs as gene regulators, oncogenic roles, and tumersupresor roles and considering this fact that miRNAs may be connected to different diseases in humans, especially to breast cancer. Therefore, the aim of the current study is to evaluate the dysregulation (up regulation and down regulation) of expressed of two different types of miRNA including miR-571 and miR-20a according to different state of chemotherapy and different grades of breast cancer and to determine the effect of the chemotherapy-drug sensitivity of breast cancer on both miR-571 and miR-20a expression .

-Objectives of the study

1. To find out the effect of chemotherapy on breast cancer patients.
2. To find out the association between grade of cancer and chemotherapy

METHOD

Collection of Samples

45 Blood samples were collected in which 40 breast cancer patients by two stages. Firstly, it included (20) samples from female cases of recently diagnosed with breast cancer at different stages in the state of pre-chemotherapy. Secondly, (20) samples from the same previewed female cases with lately diagnosed breast malignancy with varying grades of cancer stage but in the state of post-chemotherapy (patient with used chemotherapy) (same patients at two point of different time). And (5) samples as controls group. All blood samples were received in a test tube containing anticoagulant ethylene diamine tetra acetic acid (EDTA) to inhibit clotting of the blood. The blood was collected in Hiwa hospital's laboratory during six months (from October 2020 to March 2021). The required information about the patients is recorded from the patient's history files. Table 1 Collected blood samples according to different clinical features.

Table 1: Collected samples of breast Cancer (same patients at pre- and post-chemotherapy) with different cancer stages.

Clinical feature	Pre-chemotherapy	Post-chemotherapy
Stage 1	5 (12.5%)	5 (12.5%)
Stage 2	5 (12.5%)	5 (12.5%)
Stage 3	5 (12.5%)	5 (12.5%)
Stage 4	5 (12.5%)	5 (12.5%)

Extraction of miRNAs

After collecting samples, they were transported to the laboratory to extract miRNAs. For this purpose, a commercial kit from Promega Company (ReliaPrep (TM) miRNA cell and Tissue MiniPrep system lot No. 436082) was used to extract miRNAs from the plasma of blood samples. A Nano drop spectrophotometer was used to assess the quality of extracted miRNA. Extracted miRNAs were stored at -80 °C for next use.

Complementary DNA (cDNA) Preparation

All extracted miRNAs were converted to cDNA by using a commercial kit (Add Script RT Master Kit, code 22101, South Korea). Then the quality of cDNA was assessed and the quantity was measured by a Nano drop spectrophotometer and all samples were stored at -20 °C for further use.

Real Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

A qRT-PCR method did applied to assess miR-571 and miR-20a expression levels. In this method, miScript SYBR Green PCR kit is used (Qiagen) that includes miScript universal primer is used a specific forward primers in ABI PRISM 7500 real-time PCR machine (Applied Bio systems). The sequences of forward primers and Reverse primers in this study for both miRNAs was and it's were made according to the miRNA sequences that are available on the miRBase database (http://microrna.sanger.ac.uk/). PCR reaction was performed as follow:

Tube containing 20 µl including:

- 2 µl of the obtained cDNA
- 10 µl of 2× of the stain (SYBR Green)
- PCR enzyme 2 µl (Master mix)
- 10× miScript of primer known as universal primer.
- 10× miScript primer assay.

The reaction starts at 95°C for 15 min, and then the reactions was continuous for 40 cycles of 94◦C for 15s; 55◦C for 30s, and 70◦C for duration of 34s. Each reaction was run in seconds for investigation.

Statistical analysis

Collected data was analysed using SPSS v.21 software. The *chi*-square test was applied to test the differences between various studied groups at the level of P-0.05.

RESULTS

miR-571

The results of RT-PCR for expression of miR-571 (pre and post-chemotherapy and controls) samples are shown in [Table 2, 3] in which significant different was appeared. Also, there were statistically significant difference between both state of chemotherapy (pre- chemotherapy and post- chemotherapy) because the (p-value<0.05).

miR-20a

The results of RT-PCR for expression of miR-20a for (pre- and post-chemotherapy and controls) samples are provided in [Table 4,5].In which there were statistically significant difference between Pre (CT) & Post (CT) in Grade (I, II, III and IV) because p-value was less than the common alpha 0.05.

Briefly, the results of expression measurement of miR-571 and miR-20a in different patients in pre- and post-chemotherapy states are provided in [Table 6]. In which The results of the study clarified that expression of both miR-571 and miR-20a were significantly differented and changed in patients who having the first grade of the cancer before taking chemotherapy, and after chemotherapy in which p-value (<0.05), in which miR-571 was down regulated in pre-chemotherapy of all grades of patients who have breast cancer, which is meaning that the expression of miR-571 was low in cases compared with patients after treated with chemotherapy. The expression of miR-20a was nearly stable in patients with grade 2 and grade 4 of breast cancer. In contrast, the expression of miR-20a was completely different in grade 3, while in another grade, it is partially increased in a patient with chemotherapy. In these stages, increase and decrease were observed in the expression of miR-571 and miR-20a respectively.

Regards to result, the expression of both types of miRNAs (miR-571 and miR-20a) was completely different in dysregulation .for example , The expression of miR-571 was down regulated in state of pre-chemotherapy and up regulated in post-

chemotherapy state,(meaning that expression of miR571 was decreased in patients without using chemotherapy and increased in a patients when used chemotherapy). in contrast, Expression of miR-20a was up regulated in state of pre-chemotherapy and down regulated in post chemotherapy,(meaning that expression of miR20a was increased in patients without using chemotherapy and decreased in a patients when used chemotherapy). And significant change between both states occurs in all grade cancer especially in grade 4 for miR-571 and grade 3 for miR20a.

The dysregulation of both miRNAs in different stages and both pre-and post-chemotherapy is provided in Table 7 and figure (1,2) As can be seen, in grade 1 all patients showed as up regulated expression before chemotherapy for miR-20a , And after chemotherapy, 80% of patients became down regulated. In grade 2, all patients was down regulated when treated with chemotherapy. Approximately, similar results were observed in patients at grade 3. However, chemotherapy has high effect on miR-571 compared to miR20a.

Association between chemotherapy and miR-571 in breast cancer patients is shown in Table 8. From 20 investigated patients, 19 patients were positive for miR-571 before chemotherapy, but only seven were positive among those treated with chemotherapy. The statistical analysis showed a highly significant difference between patients treated with chemotherapy and those without chemotherapy.

Association between chemotherapy and miR-20a in breast cancer patients is provided in [Table 9]. Results of the study clarified that chemotherapy had a tremendous effect in curing patients suffer from breast cancer tested for miR-20a. The number of patients before treating with chemotherapy that were positive for breast cancer was 14, which was significantly more than positive patients after chemotherapy.

[Table 10] shows the difference between breast cancer patients tested for miR-571 and miR-20a. Results indicated that the number of breast cancer patients that tested for miR-571 level was significantly higher than those tested for miR-20a level ($p < 0.05$). Among 40 patients investigated in the present study, 26 patients of them showed some levels of miR-571 expression, but only 15 patients contained miR-20a in their plasma samples.

Association between breast cancer grades and expression level of miR-571 is shown in [Table 11]. The obtained results showed that the amount of miR-571 in patients having grade 4 is significantly higher than those having grades 1 to 3. As it can be seen, all patients with grade 4 showed different levels of miR-571 expression in their plasma samples.

Association between breast cancer grades and expression level of miR-20a is shown [Table 12]. There was no significant difference between negative and positive patients of breast cancer in different grades ($P > 0.05$).

Table 2: Results of RT-PCR for expression of miR-571 for (pre and post-chemotherapy) samples with significant different between both state of chemotherapy.

No.	Grade	Pre-chemotherapy (CT)	Post-chemotherapy (CT)
1	I	29	**22**
2	I	35	**20**
3	I	33	**24**
4	I	22	**17**
5	I	36	**21**
6	II	27	**21**
7	II	34	**22**
8	II	23	**19**
9	II	31	**16**
10	II	27	**19**
11	III	35	**16**
12	III	33	**21**
13	III	35	**24**
14	III	24	**21**
15	III	32	**20**
16	IV	33	**20**
17	IV	31	**18**
18	IV	31	**15**
19	IV	26	**17**
20	**IV**	**28**	**21**

*Note: The cut off of CT is 25. Positive mean less than 25 and negative mean more than 25.

Table 3: Result of RT-PCR for expression of miR-571 for controls

No of Sample	miR-571 (CT)
1	15
2	16
3	18
4	18
5	20

*Note: The cut off of CT is 25 Positive mean less than 25 Negative mean more than 25

Table4: Result of RT-PCR for expression of miR-20a (pre and post-chemotherapy) samples with significant different between both state of chemotherapy.

No.	Grade	Pre-chemotherapy (CT)	Post-chemotherapy (CT)
1	I	18.5	23
2	I	17	25.5
3	I	20	26
4	I	17.5	26.5
5	I	20	28
6	II	29	32
7	II	27	29
8	II	21	32
9	II	27	33
10	II	20	30.5
11	III	24	25.5
12	III	20	28
13	III	21.5	29
14	III	20	26
15	III	18	32
16	IV	26	26
17	IV	27	25.5
18	IV	29	28
19	IV	16.5	26
20	IV	14	27

***Note: The cut off of CT is 25. Positive mean less than 25 and negative mean more than 25.**

Table 5: Result of RT-PCR for expression of miR-20a for controls

No. of Sample	miR-20a (CT)
1	22
2	21
3	22
4	19.5
5	20

*Note: The cut off of CT is 25 Positive mean less than 25 Negative mean more than 25

Table 6: Expression of miR-571 and miR-20a in different states of chemotherapy in patients' samples.

Clinical feature	No. of samples	Sate of chemotherapy	miR-571	miR-20a
Stage 1	5	Pre-chemotherapy	-ve (80%)	+ve (100%)
Stage 1	5	Post-chemotherapy	+ve (100%)	-ve (80%)
Stage 2	5	Pre-chemotherapy	-ve (80%)	+ve (40%)
Stage 2	5	Post-chemotherapy	+ve (100%)	-ve (100%)
Stage 3	5	Pre-chemotherapy	-ve (80%)	+ve (100%)
Stage 3	5	Post-chemotherapy	+ve (100%)	-ve (100%)
Stage 4	5	Pre-chemotherapy	-ve (100%)	+ve (40%)
Stage 4	5	Post-chemotherapy	+ve (100%)	-ve (100%)

Table 7: Circulating miRNAs (miR-571, miR-20a) expressed in the plasma of different chemotherapy states and stages of breast cancer cases compared to healthy controls by RT-PCR analysis.

miRNAs	Grades	Mean Ct (Pre- chemotherapies)	Mean Ct (post-chemotherapies)	**Mean Ct (controls)**
miR-571	I	31	20.8	**17.4**
	II	28.4	19.4	**17.4**
	III	31.8	20.4	**17.4**
	IV	29.8	18.2	**17.4**
	Grades mean	**30.2**	**19.7**	**17.4**
miR-20a	I	18.6	25.8	**21.8**
	II	24.8	31.3	**21.8**
	III	20.7	28.1	**21.8**
	IV	22.5	26.5	**21.8**
	Grades mean	**21.6**	**27.9**	**21.8**

Figure 2: Illustrates miR-20a expression in (pre &post- chemotherapies and controls.

Table 8: Association between Chemotherapy and miR-571 in breast cancer patients.

Type			miR-571		Total	Significant test
			Negative	**Positive**		
General	Pre-chemotherapy	Count	17	3	20	**Chi-Square test=15.824**
		% of Total	42.5%	7.5%	50%	
	Post-chemotherapy	Count	0	20	20	
		% of Total	0%	50%	50%	**P-value<0.05**
	Total	Count	17	23	40	
		% of Total	42.5%	57.5%	100%	

Table 9: Association between Chemotherapy and miR-20a in breast cancer patients.

Type			miR-20a		Total	Significant test
			Negative	**Positive**		
General	Pre-chemotherapy	Count	6	14	20	**Chi-Square test=18.027**
		% of Total	15.0%	35.0%	50%	
	Post-chemotherapy	Count	19	1	20	
		% of Total	47.5%	2.5%	50%	**P-value< 0.05**
	Total	Count	25	15	40	
		% of Total	62.5%	37.5%	100.0%	

Table 10: Difference between the level of miR-571 and miR-20a in breast cancer patients.

miRNA		**Negative**	**Positive**	**Total**
miR-571	Count	17	23	40
	% of Total	21.25%	28.75%	50%
miR-20a	Count	25	15	40
	% of Total	31.3%	18.8%	50%
Total	Count	42	38	80
	% of Total	52.5%	47.5%	100%
Significant test	Chi-Square test = 6.054		P-value = 0.014	

Table 11: Association between breast cancer grades and expression level of miR-571.

Grade		**miR-571**		**Total**
		Negative	**Positive**	
1	Count	4	6	**10**
	% of Total	10.0%	15.0%	**25.0%**
2	Count	4	6	**10**
	% of Total	10.0%	15.0%	**25.0%**
3	Count	4	6	**10**
	% of Total	10.0%	15.0%	**25.0%**
4	Count	5	5	**10**
	% of Total	12.5%	12.50%	**25.0%**

Total	Count	17	23	**40**
	% of Total	42.5%	57.5%	**100.0%**
Significant test	Chi-Square test = 8.352		**P-value = 0.039**	

Table 12: Association between breast cancer grades and expression level of miR-20a.

Grade		**miR-20a**		**Total**
		Negative	**Positive**	
1	Count	4	6	**10**
	% of Total	10.0%	15.0%	**25.0%**
2	Count	8	2	**10**
	% of Total	20.0%	5.0%	**25.0%**
3	Count	5	5	**10**
	% of Total	12.5%	12.5%	**25.0%**
4	Count	8	2	**10**
	% of Total	20.0%	5.0%	**25.0%**
Total	Count	25	15	**40**
	% of Total	62.5%	37.5%	**100.0%**
Significant test	Chi-Square test = 5.44		**P-value = 0.142**	

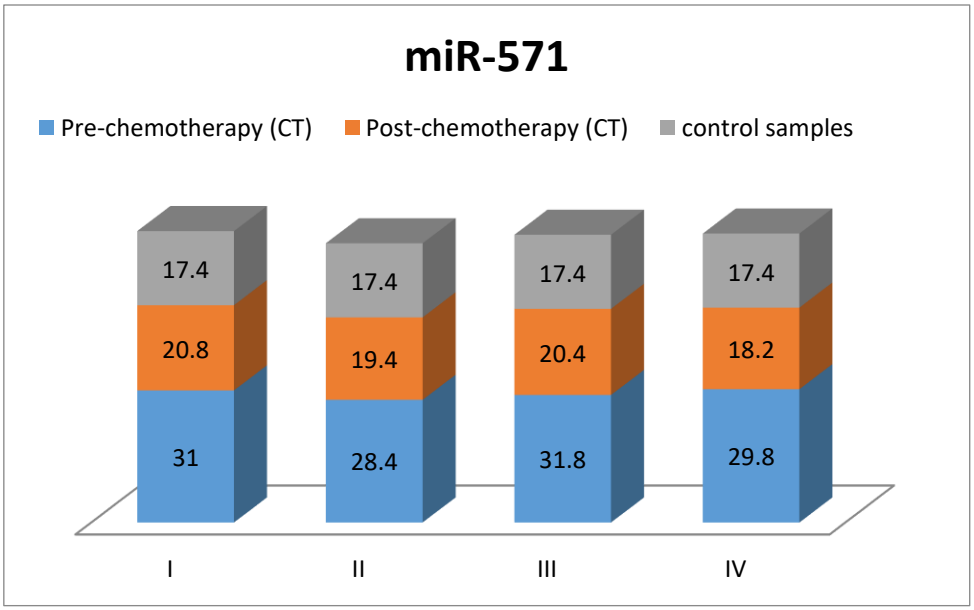

Figure 1: shows miR-571 expression in pre &post- chemotherapies and controls.

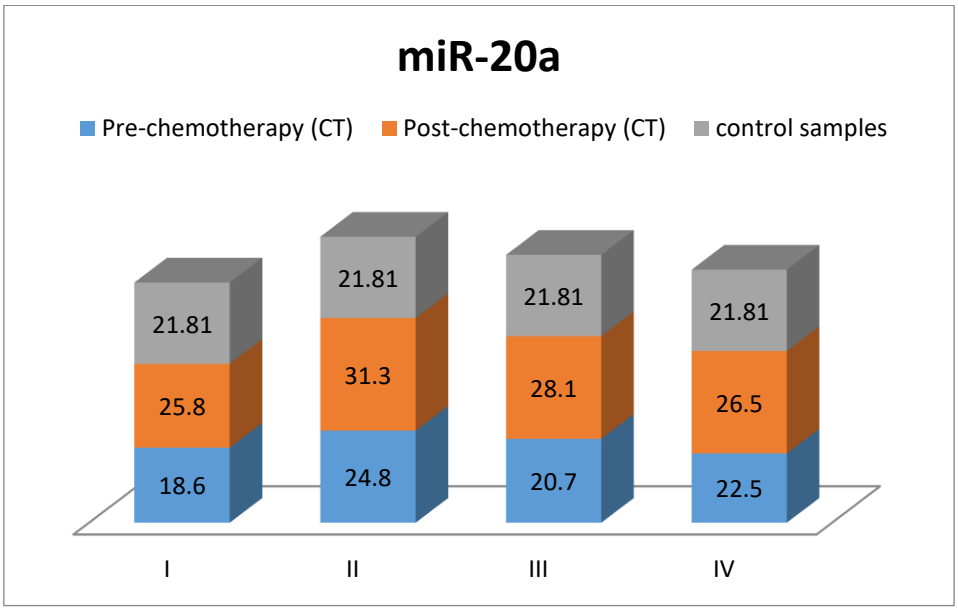

Figure 2: Illustrates miR-20a expression in (pre &post- chemotherapies and controls.

DISCUSSION

The relationships between miRNAs and breast cancer have been broadly studied, giving significantly to investigations of breast cancer pathogenesis and their clinical implications. These short molecules play important roles in the oncogenesis, growth, invasion, metastasis, and angiogenesis of breast cancer; thus, altered expression of miRNAs can be regarded as a target for the diagnosis and/or treatment of breast cancer based on the miRNA expression profile(Calin et al .,2006). miRNAs have newly appeared as giving specific biomarkers for disease situations in a number of cancer patients as well as in other conditions, due to their stability and facility of discovery(Chen et al,.2008 :Creemers et al.,2012). In this research, the differential miRNAs expression in breast cancer patients with different grades and states of chemotherapy in Hiwa hospital were studied. Some particular miRNAs defined to a special group may be completely correlated with their clinical presentation.miRNAs were highly expressed in breast cancer patients with pre-chemotherapy in different grades. In contrast, less miRNA dysregulation was found in breast cancer patients with post-chemotherapy in any grades of breast cancer. In other words, the quantity of miRNAs expression is high in breast cancers with pre-chemotherapy compared with post-chemotherapy. Chemotherapy is the modern therapeutic technique that is being used for breast cancer, especially for triple-negative forms, but the patients do not usually receive a desirable outcome. miRNAs can suggest a new alternative therapeutic method for yielding better breast cancer chemotherapy outcome (Amir Mehrgou and Mansoureh Akouchekian, 2017).

Currently, miRNA is not only a predictor of chemo resistance and determination of grades, but circulating miRNA could also be used as a biomarker for cancer detection and monitoring as well as subtype prediction (Hao Wang et al., 2018).

Blood-based diagnostics, such as detecting circulating miRNAs in plasma, might be a valuable addition to current diagnostic techniques, allowing for better breast cancer screening and diagnosis. The present study aims to estimate the quantitation of the miR-571, miR-20a in the blood plasma of people who certainly diagnosed having breast cancer after common treatments such as chemotherapy using RT qPCR.

Our result showed that the expression of (miR-20a) as being significantly up-regulated in the plasma of breast cancer patients in the state of pre-chemotherapy ($p < 0.05$) meaning that the expression of this miRNA was increased in the cases of BC without using any treatment. Several recent studies have demonstrated that the expression of (miR-20a) was up regulated in the plasma of breast cancer patients without using chemotherapy (Chen and Wang, 2014: Niuet al., 2013).Also, miRNA (miR-571) has been reported as down regulated ($p < 0.05$) in the state of pre-chemotherapy, meaning the expression of this miRNA was decreased in the pre-chemotherapy of BC patients.

A recent study has identified miR-571 to be more than 1.5 fold down regulated in breast cancer patients (Brase et al., 2010). In addition: Based on the outcomes obtained in our study, among two expressed miRNA in breast cancer patients, miR-571 is the first miRNA in breast cancer that has a high level of expression and high rate of sensitive to chemotherapy compared with others. some were extensively studied since their initial discovery and revealed an important role in the biology of breast cancer, overexpressed in breast cancer, has been demonstrated to mediate cell survival and proliferation directly targeting the oncosuppressor genes PTEN, PDCD4, and TPM1, and it has been associated by excellent clinical stage, lymph node metastasis and poor patient prognosis (Yan LX et al., 2008: Qian et al., 2008). Chemotherapy is the current therapeutic method that is being used for breast cancer, especially for triple-negative forms, but the patients do not usually receive a desirable outcome. miRNAs can suggest a new alternative therapeutic method for yielding better breast cancer chemotherapy outcome.

Researches have revealed that the expression level of miRNAs can correlate to patients' response to chemotherapy. Modern studies recommended that up-regulation of some miRNAs occurs in breast cancer and relates to chemo resistance (Hu et al., 2013). According to our result, among two expressed miRNAs in post-chemotherapy of breast cancer miR-571 is the only miRNA that has a high level of expression, meaning it became up regulated in BC patients with treated chemotherapy; up-regulation of miR-571 contributes to an increased response to drug therapy. In contrast, the expression of (miR-20a) are down regulated in post-chemotherapy, and it meaning that the drug is high sensitive and effect on patients or decreased the expression of these miRNAs may be signature for patient therapy outcome. Studies have shown that miRNAs can have a role in drug resistance. miRNAs cause a reduction of drug resistance and are downregulated in progressed breast (Li XFet al., 2009).

The results of the present study showed that all patients with grade 4 of breast cancer had miR-571 in their plasma samples, indicating the overexpression of this miRNA in the late stages of breast cancer. However, this was not observed for miR-20a. In addition to the above mentioned results.

The present study suggested that chemotherapy has more effects on miR-571 expression compared to miR-20a, and this effect is more observed in the early stages of developing breast cancer than

other stages. In addition, both miRNAs investigated in this study can be considered as putative breast cancer markers for detecting chemotherapy's effect on patients.

CONCLUSIONS

Differential miRNAs were expressed in both control groups and cases of breast cancer. miR-20a was up-regulated in the cases of breast cancer in the state of pre-chemotherapy. miR-571 was down regulated in the cases of breast cancer with the state of pre-chemotherapy.) miR-571 can be used as a biomarker in breast cancer diagnosis and moitoring. miRNA is a predictor of chemo resistance and chemo sensitivity, and circulating miRNA could be utilized as a biomarker for early cancer detection .The applicability of miRNAs as biomarkers of rapid cancer growth may provide the improvement of precision medicine and improve life expectancy and quality of life from affected patients. Chemotherapy has more effect on dysregulation of miRNAs than variability in grades of breast cancer.

ETHICAL CONSIDERATIONS COMPLIANCE WITH ETHICAL GUIDELINES

An administrative agreement was obtained from College of Science, Biology Department, University of Sulaimani, Iraq. The participants were informed about the research's purpose and ensured anonymity and confidentiality of the information. A written informed, voluntary participation consent was obtained from each participant.

FUNDING

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

AUTHOR'S CONTRIBUTIONS

Study concept; Writing the original draft;Data collection; Data analysis and Reviewing the final edition by all author.

DISCLOSURE STATEMENT: The authors report no conflict of interest.

ACKNOWLEDGEMENTS

We thank the anonymous referees for their useful suggestions.

REFERENCES

A. Bahrami, A. Aledavood, K. Anvari, S.M. Hassanian, M. Maftouh, A. Yaghobzade, O. Salarzaee, S. ShahidSales, A. Avan,.,2018. "The prognostic and therapeutic application of microRNAs in breast cancer: Tissue and circulating microRNAs," Journal of Cellular Physiology, 233, pp. 774–786.

A. J. Murray, D. M. Davies., 2013. "The genetics of breast cancer," Surgery, 31 (1), pp. 1-3.

Amir Mehrgou and Mansoureh Akouchekian., 2017. Therapeutic impact of microRNAs in breast cancer by their roles in regulating processes involved in this disiease, Cell research 18, 997–1006.

Brase JC, Johannes M, Schlomm T., 2010. Circulating miRNAs is correlated with tumor progression in prostate cancer. Int J Cancer; 128:608–16.

C. Jackisch, N. Harbeck, J. Huober, G. von Minckwitz, B. Gerber, H. H. Kreipe, et al., 2015. "14th St. Gallen International Breast Cancer Conference 2015: Evidence, Controversies, Consensus - Primary Therapy of Early Breast Cancer: Opinions Expressed by German Experts," Breast Care (Basel, Switzerland), 10 (3), pp. 211–219.

C. Zhou, G. Li, J. Zhou, N. Han, Z. Liu, J. Yin., 2014. "miR-107 activates ATR/Chk1 pathway and suppress cervical cancer invasion by targeting MCL1," Plos One, 9, (11), e111860.

Calin, G. A., & Croce, C. M., 2006. MicroRNA signatures in human cancers. In *Nature Reviews Cancer* ,Vol. 6, Issue 11.

Chen J, Wang X., 2014. MicroRNA-21 in breast cancer: diagnostic and prognostic potential. *Clinical & translational oncology: official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico*; 16(3):225–33.

Chen, X., Ba, Y., Ma, L., Cai, X., Yin, Y., Wang, K., Guo, J., Zhang, Y., Chen, J., Guo, X., Li, Q., Li, X., Wang, W., Zhang, Y., Wang, J., Jiang, X., Xiang, Y., Xu, C., Zheng, P., … Zhang, C. Y., 2008. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Research*, *18*(10).

Creemers, E. E., Tijsen, A. J. & Pinto, Y. M., 2012. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? Circulation research 110, 483–495.

D. Hamam., 2014. "MicroRNA-320/RUNX2 axis regulates adipocytic differentiation of human mesenchymal (skeletal) stem cells," Cell death & disease, 5, p.p. e1499.

E. C. Lai., 2003. "microRNAs: Runts of the genome assert themselves," Current Biology, 13, pp. 925–936.

G. Curigliano, H. J. Burstein, E. P. Winer, M. Gnant, P. Dubsky, S. Loibl, et al., 2017. "De-escalating and escalating treatments for early-stage breast cancer: The St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer," Annals of Oncology, 28, pp. 1700–1712.

Hao Wang, Ran Pang, Juniie Wang, Zelian Qin Liaxiang Xue., 2018. Circulating microRNAs as potential cancer biomarkers: the advantage and dis advantage.

He L, Hannon GJ., 2004. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet*5:522–31. doi: 10.1038/nrg1379.

J. Cui, J. Mo, M. Luo, Q. Yu, S. Zhou, T. Li, et al., 2015. "c-Myc-activated long non-coding RNA H19 down regulates miR-107 and promotes cell cycle progression of non-small cell lung cancer," International Journal of Clinical and Experimental Pathology, 8, pp. 12400-12409.

K. H. Lee, C. Lotterman, C. Karikari, N. Omura, G. Feldmann, N. Habbe, et al., 2009. "Epigenetic silencing of microRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer," Pancreatology, 9, pp. 293-301.

Katarina Cuk, Manuela Zucknick , Jorg Heil, et al., 2013. "Circulating microRNAs in plasma as early detection markers for breast cancer," 1602–1612 .

L. Feng, Y. Xie, H. Zhang, Y. Wu., 2012. "miR-107 targets cyclin-dependent kinase 6 expression, induces cell cycle G1 arrest and inhibits invasion in gastric cancer cells," Medical Oncology, 29, pp. 856-863.

L. He, G. J. Hannon., 2004. "MicroRNAs: small RNAs with a big role in gene regulation," Nature Reviews. Genetics, 5, p.p. 522–531.

Li XF, Yan PJ, Shao ZM., 2009. Down regulation of miR-193b contributes to enhance urokinase-type plasminogen activator (uPA) expression and tumor progression and invasion in human breast cancer. Oncogene; 28:3937-48.

Li XF, Yan PJ, Shao ZM., 2018. Downregulation of miR-193b contributes to enhance urokinase-type plasminogen activator (uPA) expression and tumor progression and invasion in human breast cancer. Oncogene; 28:3937-48, 2009. X. Bai, G. Han, Y. Liu, H. Jiang, Q. He, "MiRNA-20a-5p promotes the growth of triple-negative breast cancer cells through targeting RUNX3," Biomedicine & Pharmacotherapy, 103, pp. 1482-1489.

M. A. Aleskandarany, M. E. Vandenberg he, C. Marchiò, I. O. Ellis, A. Sapino, E. A. Rakha., 2018. "Tumour Heterogeneity of Breast Cancer: From Morphology to Personalised Medicine," Pathobiology, 85 (1-2), pp. 23-34.

M. Sochor, 2014. "Oncogenic microRNAs: miR-155, miR-19a, miR-181b, and miR-24 enable monitoring of early breast cancer in serum," BMC Cancer, 14, p.p. 448.

Niu J, Shi Y, Tan G, Yang CH, Fan M, Pfeffer LM., 2012. DNA damage induces NF-kappaB-dependent microRNA-21 up-regulation and promotes breast cancer cell invasion. *The Journal of biological chemistry*;287(26):21783–95.

P. S. Chen, J. L. Su, S. T. Cha, W. Y. Tarn, M. Y. Wang, H. C. Hsu, et al., 2017. "miR-107 promotes tumor progression by targeting the let-7 microRNA in mice and humans," Journal of Clinical Investigation, 127 (3), pp. 1116.

P. Sharma, A. Saraya, P. Gupta, R. Sharma., 2013. "Decreased levels of circulating and tissue miR-107 in human esophageal cancer," Biomarkers, 18, pp. 322-330.

Qian B, Katsaros D, Lu L, Preti M, Durando A, Arisio R., 2008. High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF-beta1. Breast Cancer Res Treat Oct.Hu H, Li S, Cui X, Lv X, Jiao Y, Yu F., 2013. The overexpression of hypomethylated miR-663 induces chemotherapy resistance in human breast cancer cells by targeting heparin sulfate proteoglycan 2 (HSPG2) *J Biol Chem; 288:10973*–85.

S. K. Chia, V. H. Bramwell, D. Tu, L. E. Shepherd, S. Jiang, T. Vickery, et al., 2012. "A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen," Clinical cancer research: an official journal of the American Association for Cancer Research, 18 (16), p.p. 4465–4472.

S. Kurozumi, Y. Yamaguchi, M. Kurosumi, M. Ohira, H. Matsumoto, J. Horiguchi., 2017. "Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes," Journal of Human Genetics, 62, pp. 15-24.

S. Volinia, G. A. Calin, C. G. Liu, S. Ambs, A. Cimmino, F. Petrocca, et al., 2006. "A microRNA

expression signature of human solid tumors defines cancer gene targets," Proceedings of the National Academy of Sciences of the United States of America, 103 (7), p.p. 2257–2261.

S. Wang, G. Ma, H. Zhu, C. Lv, H. Chu, N. Tong, et al., 2016. "miR-107 regulates tumor progression by targeting NF1 in gastric cancer," Scientific Reports, 6, 36531.

Y. Sun., 2012. "Serum microRNA-155 as a potential biomarker to track disease in breast cancer," PloS One, **7**, p.p. e47003.

Y. Takahashi, A. R. Forrest, E. Maeno, T. Hashimoto, C. O. Daub, J. Yasuda., 2009. "miR-107 and miR-185 can induce cell cycle arrest in human non-small cell lung cancer cell lines," Plos One, 4, e6677.

Y. Wang, F. Chen, M. Zhao, Z. Yang, S. Zhang, L. Ye, et al., 2016. "miR-107 suppresses proliferation of hepatoma cells through targeting HMGA2 mRNA 3'UTR," Biochemical and Biophysical Research Communications, 480, pp. 455-460.

Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL., 2008. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA Nov;14:2348–60.