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# Role of microRNAs -29b-2, −155, −197 and −205 as diagnostic biomarkers in serum of breast cancer females☆,☆☆

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ABSTRACT

Micro-RNAs (miRs) are known to be differentially expressed in the serum of cancer patients and controls, and can thus be used as biomarkers for cancer screening. We detected the expression level of miR-29b-2, 155, 197 and 205 in the serum of female breast cancer patients and healthy controls to detect whether serum level of this chosen micro-RNAs could detect patients with breast cancer and also to detect difference in level of micro-RNAs between non-metastatic cases and metastatic cases, also we tried to detect any relation between level of micro-RNAs and the stage of the tumor, the size of tumor, nodal affection, the presence of metastasis and also the site of metastasis. Serum samples were collected from 130 female patients, 80 with non-metastatic breast cancer, 20 with metastatic breast cancer and 30 healthy controls. Real-time quantitative PCR was used to detect the expression level of miR-29b-2, −155, −197 and −205. The expression level of miR-29b-2, −155, −197 and −205 was significantly increased in the serum of breast cancer patients. miR-29b-2, −155, −197 and −205 may be useful as a blood-based biomarker for breast cancer screening.



## 1. Introduction

Breast cancer is the most common female malignancy around the world. It was reported that the expected numbers of new breast cancer cases in 2012 is 230,480, which is expected to account for 30% of all new cancer cases among women (Siegel et al., 2012).

Breast cancer is the second leading cause of cancer-related mortality, after lung cancer, in women all over the world. Almost one of every three affected women will die from the disease (Benson et al., 2009). The incidence of breast cancer varies greatly around the world; it is lowest in less-developed countries and greatest in the more-developed ones (Stewart and Kleihues, 2003). Breast cancer in Egyptian patients is biologically more aggressive disease than that encountered in the West. This may be explained partly by the predominance of premenopausal patients and partly by the late presentation of patients at an advanced stage (El-Bolkainy, 2000).

Micro-RNA is a small non-coding RNA molecule containing about 22 nucleotides found in plants, animals, and some viruses, which have role in transcription and post-transcription regulation of gene expression (Chen and Rajewsky, 2007).

The human genome may encode over 1000 micro-RNAs, which may target about 60% of mammalian genes (Bentwich et al., 2005). Micro-RNAs are well conserved in eukaryotic organisms and are thought to be a vital and evolutionarily ancient component of genetic regulation (Kren et al., 2009).

It is well established that micro-RNAs represent an important determinant of global messenger RNA (mRNA) expression in normal and diseased tissue (Bushati and Cohen, 2007), including cancers (Cho, 2007). Because a single micro-RNA can regulate the expression of multiple proteins, micro-RNAs are thought to be a better diagnostic parameter than messenger RNAs and a more effective target of selective therapeutic modalities.

Still little is known about the role of micro-RNAs in development of normal breast tissue, so this study is aiming to detect level of expression of miR-29b-2, −155, −197 and −205 in the serum of breast cancer females in contrast to control group, and their relation to nodal and distant metastasis.

Many mammalian genomes encode four closely related miR-29 precursors that are transcribed in two transcriptional units. For example, human miR-29a and miR-29b-1 are processed from an intron of a long non-coding transcript (LOC646329) from chromosome 7. MiR-29b-2 (identical in sequence to miR-29b-1) and miR-29c are co-transcribed from chromosome 1. The three main mature micro-RNAs processed from these precursors are known as hsa-miR-29a, hsa-miR-29b, and hsa-miR-29c (Chang et al., 2008).

The mature products are thought to exert regulatory roles by binding with partial complementarity to micro-RNA recognition elements

*Abbreviations:* miRs, Micro-RNAs; PCR, polymerase chain reaction; RNA, ribonucleic acid; mRNA, messenger RNA; MREs, micro-RNA recognition elements; UTR, untranslated region; TCL1A, T-cell leukemia/lymphoma.

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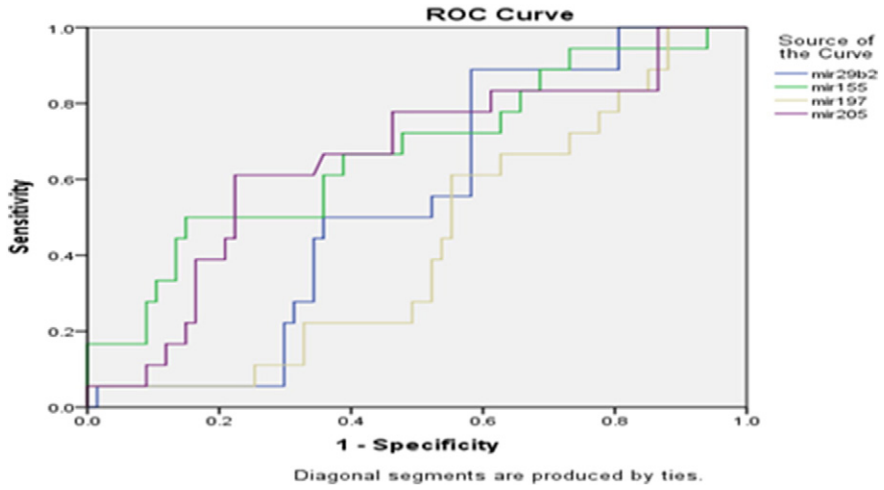


**Fig. 1.** ROC curve for all micro-RNAs to detect metastasis (Values below cutoff values are metastatic).

(MREs) in the 3′ untranslated region (3′ UTR) of target transcripts. Experimental evidence suggests that targets of mature miR-29 products include the following, the myeloid leukemia cell differentiation protein (MCL1), an anti-apoptotic member of the Bcl-2 family of proteins (Mott et al., 2007). The TCL1A (T-cell leukemia/lymphoma 1) oncogene is found to be disrupted in many T-cell leukemias (Pekarsky et al., 2006). DNA methyltransferases (DNMT3A) and (DNMT3B), which are frequently upregulated in lung cancer (Fabbri et al., 2007). Zinc finger protein 36 homolog (ZFP36), also known as tristetraprolin (TTP), an anti-inflammatory and anti-cancer gene (Sanduja et al., 2011).

The miR-29 family of microRNAs, including miR-29a, miR-29b and miR-29c, was recently reported to be upregulated in multiple cancers. Increasing evidence shows that the abnormal expression of miR-29 family is associated with tumorigenesis and cancer progression, making miR-29s a well-analyzed group of micro-RNAs in cancer research (Wang et al., 2013).

miR-155 has many clinical importance as it may play a role in hypertension and cardiovascular diseases (Faraoni et al., 2009), miR-155 also involved in immunity as it has humoral and innate cell-mediated immune responses. Also it plays a role in inflammatory process, miR-155 is overexpressed in atopic dermatitis and contributes to chronic skin inflammation by increasing the proliferative response of T (H) cells (Sonkoly et al., 2010).

miR-197 was identified as most prominently up-regulated in male breast cancer (Lehmann et al., 2010). Little is known about mir-197 and this is the first research done to detect its level of expression in female breast cancer cases.

Members of miR-200 family including miR-205 are found clustered at two locations in the human genome: 1142000–1144500 in chromosome 1 and 6942000–6944500 in chromosome 12. Short genomic distance between members suggests that they may function collaboratively and are highly related in sequence (Gregory et al., 2008).

Studies have demonstrated that miR-205 has a role in both normal development and cancer.

## 2. Subjects and methods

The present study was conducted on 130 female subjects aged from 25 to 75. They were classified into 3 groups: Group 1: control; 30 healthy females with no family history of breast cancer, or any other diseases. Group 2: nonmetastatic breast cancer patients with only nodal affection; 80 female patients. Group 3: metastatic breast cancer patients with nodal affection and distant metastasis (bone, liver and lung); and 20 female patients.

Informed written consent from each patient and local ethical committee approval was available before starting data collection. With respect to patients' confidentiality, patients were represented in the study by code numbers and not by their names with all personal data concealed. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from the participants in this study after ethical committee approval from Medical Biochemistry Department, Faculty of Medicine Cairo University.

### 2.1. Sample collection and serum processing

Informed consent was obtained from the participants in this study after ethical committee approval from Medical Biochemistry Department, Faculty of Medicine Cairo University. Blood samples were collected from 130 women including 100 breast cancer patients and 30 healthy females as normal controls, their age ranged between 25 and 75 years. The patients were diagnosed by mammography, U/S, and pathological examination of tissue biopsy. Serum is separated from blood sample and stored at −80 °C.

### 2.2. RNA extraction

Total RNA with preserved micro-RNAs was extracted from 200 μL serum by miRNeasy extraction kit (Qiagen, Valencia, CA, USA) using 1000 μL QIAzol lysis reagent and incubated for 5 min at RT. Then, 200 μL chloroform was added, vortexed for 15 s, and incubated for

**Table 1**
Pathological data in all patients.

| | | All patients | | Groups | | | |
|---|---|---|---|---|---|---|---|
| | | | | Nonmetastatic breast cancer | | Metastatic breast cancer | |
| | | Count | % | Count | % | Count | % |
| Type of tumor | Invasive duct ii | 81 | 81% | 69 | 85.5% | 12 | 60.0% |
| | Invasive duct iii | 19 | 19% | 11 | 14.5% | 8 | 40.0% |
| T | T2 | 74 | 74% | 58 | 72.5% | 16 | 80.0% |
| | T3 | 26 | 26% | 22 | 27.5% | 4 | 20.0% |
| N | N2 | 78 | 78% | 63 | 78.8% | 15 | 75.0% |
| | N3 | 22 | 22% | 17 | 21.3% | 5 | 25.0% |
| M | 1 | 20 | 20% | 0 | 0.0% | 20 | 100.0% |
| | Zero | 80 | 80% | 80 | 100.0% | 0 | 0.0% |
| U/S | Fatty liver | 76 | 76% | 64 | 80.0% | 12 | 60.0% |
| | Hcc | 5 | 5% | 0 | 0.0% | 5 | 25.0% |
| | Normal | 19 | 19% | 16 | 20.0% | 3 | 15.0% |
| Chest x-ray | Lung met | 15 | 15% | 0 | 0.0% | 15 | 75.0% |
| | Normal | 85 | 85% | 80 | 100.0% | 5 | 25.0% |
| Bone scan | Bone mtast | 3 | 3% | 0 | 0.0% | 3 | 15.0% |
| | Normal | 97 | 97% | 80 | 100.0% | 17 | 85.0% |