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ASSESSMENT OF MICRORNA-182 AND MICRORNA-133 AS NON-INVASIVE PREDICTORS OF BREAST CANCER IN PAKISTAN

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Abstract

Background: Breast cancer is a diverse heterogeneous disease and it has become difficult to track the progression of breast cancer. It has been reported that miRNAs play a role in therapeutic targets and diagnostic markers.

Methods: In the study, the expression levels of miRNA-182, and miRNA-133 were assessed of total 292 patients through purposive sampling in one of the public hospitals of Pakistan. Out of which 165 were cases and 127 were controls.

Results: The results of the study revealed that in both groups the levels of miRNA-182 and miRNA-133 expression levels were assessed. Hence the expression levels of miRNA-182, in both groups were similar in terms of age, status of menopause, and BMI. The patients suffering from breast cancer expressed more miRNA-133 than the control group did, and there were notable differences between the two groups when it came to older age, status of menopause, and BMI (obesity). A sensitivity of 94.1%, specificity of 100%, positive predictive value of 100 %, and negative predictive value of 80.81% were obtained for miRNA-133 levels. These findings suggested that miRNA-133 possessed strong predictive power. A sensitivity of 74.52%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 59.38% were obtained for miRNA-182.

Conclusion: The study concludes that the function of miRNA-182 and miRNA-133 are considered As Non-Invasive Predictors in the course and outcome of Breast Cancer.

Keywords:-Breast cancer, heterogenous disease, miRNA, non-invasive predictors

Introduction

Global Public Health statistics showed that over 410,000 and over a million women are diagnosed with breast cancer annually (1). Breast cancer (BC) accounted for approximately 25% of all newly diagnosed cases of cancer in 2012, making it the second most common cancer worldwide and the most common cancer among women (2). It is a diverse heterogeneous disease with a wide range of morphological characteristics and behaviors (3). MicroRNAs are non-coding RNA molecules with a length of roughly 17–25 highly conserved nucleotides. Through their interaction with a particular target messenger RNA (mRNA), they regulate the expression of genes at the post-transcriptional level. Traditionally, miRNAs can directly influence genic expression through intracellular mechanisms or indirectly through micro-vesicle release, thereby enabling the regulation of gene expression across diverse tissues (4). A wide range of cellular functions, including differentiation, aging, metabolism, proliferation, and cell death, are also regulated by them. In particular, multiple studies have discovered that miRNAs are essential for controlling cell proliferation, differentiation, apoptosis, and carcinogenesis (5,6). As a result, nearly every area of biology and biomedicine is beginning to acknowledge the significance of miRNAs (7). Currently, the human genome contains more than 2500 mature miRNAs that have been identified and cataloged, controlling about 30% of all protein-coding genes (8,9). Tissue samples and biological fluids (such as serum, plasma, urine, saliva, sweat, and tears) can be used to detect both intra and extracellular miRNAs; however, this technique is currently underutilized in personalized medicine as a diagnostic or therapeutic tool (10).

The management of breast cancer depends on an accurate early diagnosis. Despite the widespread use of mammography screening to detect breast cancer, there are significant rates of false-positive and false-negative results and mammography radiation has always been a source of concern (11). There are several limitations in vivo diagnostic tools such as mammography and ultrasound, such as breast density or calcification recognition, which were used to detect breast cancer at an early stage. In the previous 20 years, notable advancements in the hunt for non-invasive biomarkers for breast cancer have evolved (12).

Hence, miRNA is stable in serum and has significant roles in oncogenesis which has created new avenues for the early detection of cancer (13). Hence, miRNA has the potential to be extremely helpful as a new biomarker for breast cancer that can be used for each patient (14). However, the specific mRNA target of the dysregulated miRNAs in breast cancer remains unclear. In this study, research has been done on the potential of miRNAs like miRNA-182 and miRNA-133, as non-invasive predictors in the context of breast cancer.

Material & Methods

In the study, the expression levels of miRNA-182, and miRNA-133 were assessed on a total of 292 female patients through purposive sampling in one of the public hospitals of Karachi, Pakistan between March and August 2023. Out of which 165 were cases and 127 were controls. The Institutional Review Board (IRB) of the hospital approved the study. All the patients were informed regarding the objectives of the study and written consent was taken from all the patients. Each patient was asked to perform the following tests such as hematological and biochemical tests, imaging diagnosis, and a chest x-ray for stage IV. Before beginning any treatment or surgery, samples were taken from every patient. The age of participants revealed a mean ± SD of 42.9 ± 9.55 years in control females and 50.4 ± 7.54 years in breast cancer patients. Two serum collection tubes were filled with seven ml of blood, which was then centrifuged for 20 minutes at 8000 RPM after being left to clot for 40 minutes. The serum that was yielded was separated into two microtubes and kept at -100 ºC until it was analyzed.

The manufacturer's instructions were followed when using the miScript SYBR Green PCR Kit (200) from Qiagen, Germany (Catalogue no. 218073) for quantitative real-time PCR. Step One (Applied Biosystems, USA) was utilized to quantify the levels of miRNA expression. The cycling program consists of a preliminary activation step that lasts for 15 minutes at 95°C to stimulate HotStarTaq DNA polymerase, followed by cycling that lasts for 30 seconds at 55°C, 15 seconds at 94°C, and 30 seconds at 70°C, where fluorescence data collection is done (15).

Statistical analysis

To conduct statistical analysis for the current study, SPSS software (version 23) was utilized. One sample was subjected to Wilcoxon's rank sum test to compare the expression of miRNA in cancer to that in normal serum. The Mann-Whitney U non-parametric test was used for comparison between metastatic and non-metastatic. A correlation between two variables was determined by computing Spearman's rho (r). Less than 0.05 p-values were statistically significant.

Results

The results of the study revealed that miRNA-182 and miRNA-133 expression levels were assessed using Quantitative Real-time Polymerase Chain Reaction**.** There was a significant difference in the expression levels of miRNA-182 and miRNA-133 between BC serum and healthy controls ($p < 0.001$) and breast cancer serum and healthy controls ($p < 0.001$). The two miRNAs were found to be able to distinguish breast cancer from healthy controls in ROC curve analysis, with an area under the curve (AUC) of 0.949 for miRNA-133 (95% CI 0.88-1.00, $p < 0.001$) and 0.766 (95% CI 0.65-0.86, $p < 0.001$) for miRNA182. The sensitivity of miRNA-133 is 94.1% and specificity is 100% and for miRNA-182 sensitivity is 74.52% and specificity is 100%, respectively. The ROC curve results indicated that serum miRNA-133 had higher diagnostic efficacy than serum miRNA-182, with AUCs of 0.949 and 0.766, respectively, and total accuracy of 97.3% and 81.5% as shown in Table 2. The clinical pathological data of the breast cancer patients revealed that the Patients with grade (III) tumors had significantly lower levels of miRNA-133 in their sera than patients with grade II tumors (P=0.041). Additionally, there was a statistically significant correlation (P=0.023) between miRNA-182 and lymph node involvement.

Table 1 Association between different clinicopathological characteristics and research markers in breast cancer patients**.**

	Relative expression of P-valued microRNAs in various groups				**The relative concentration of P-valued microRNAs in various groups**	
Variable	**miRNA-133**	**P value**	**miRNA-182**	**P value**	**p 53**	**P value**
Age ≤50 >50	-1.46 -1.80	0.560	2.23 2.62	0.951	6.57 6.60	0.773
Menopausal status PRE POST	-1.52 -1.86	0.656	2.30 2.11	0.690	6.51 5.59	0.891
Stages I II III IV	-1.21 -1.75 -0.90 -2.11	0.221	1.90 2.90 1.13 1.62	0.570	6.51 6.54 6.42 6.60	0.512
Grades II III	-1.40 -2.10	0.041*	2.90 1.20	0.104	6.54 6.63	0.651

ER(IHC) +VE -VE	 -1.76 -1.12	 0.371	 2.21 2.01	 0.810	 6.61 6.41	 0.181
PR(IHC) +VE -VE	 -1.71 -1.39	 0.521	 2.10 2.61	 0.989	 6.52 6.49	 0.369
Lymph node involvement NO YES	 -1.86 -1.51	 0.542	 1.41 3.41	 0.023*	 6.62 6.51	 0.940

- Immunohistochemistry (IHC)

Table 2 Efficacy of miRNAs and P53

	AUC	95% CI (SE)	Sensitivity	Specificity	Cut-off	P-value	PPV	NPV	TA
miRNA-182	0.766	0.65-0.86 (0.053)	74.52%	100%	1.005	0.000	100%	59.38%	81.5%
miRNA-133	0.949	0.88-1.00 (0.026)	94.1%	100%	0.939	0.000	100%	80.81%	97.3%
p53	0.731	0.590-0.871 (0.070)	54%	85%	6.890	0.002	55%	80%	76.5%

- Positive predictive value (PPV)
- Negative predictive value (NPV)
- Total accuracy (TA)

Table 3 Associations between the concentrations of p53 and miRNA expression in the serum of patients with breast cancer

	miRNA-133	miRNA-182	P53
miRNA-133 **CC** **P value**	1.01	.243 0.58	 .168 .195
miRNA-182 **CC** **P value**	 .243 0.58	 1.01	 .013 .915
P53 (ng/ml) **CC** **P value**	 .168 .195	 .013 .915	 1.01

- Spearman's correlation coefficient (CC)

Discussion

The pathophysiology of cancer is significantly influenced by microRNAs. Numerous pathways, such as the cell cycle, angiogenesis, invasion, and metastasis, can be linked to tumorigenesis by either up or down-regulating oncogenic miRNAs or tumor suppressor miRNAs (16). Therefore, it remains imperative to clarify the novel mechanism of breast cancer development to create an accurate and affordable screening method for this disease. The discovery of microRNAs, which are small non-protein-coding RNAs and have significant roles in oncogenesis, has recently created new paths for the early detection of cancer (17).

In the study, the expression levels of miRNA-182, and miRNA-133 were assessed of total 292 patients. Out of which 165 were cases and 127 were controls. One non-coding RNA called miRNA-133 plays a key role in reducing the advancement of breast cancer because its expression is lost in breast cancer patients, which is linked to abnormal cell invasion and proliferation and a poor prognosis (18). Hence, miRNA-133 controls the cell cycle and proliferation of breast cancer cells by focusing on the epidermal growth factor receptor (EGFR) through the EGFR/Akt signaling pathway or it targets fascin actin-bundling protein 1 (19, 20). The results of the study revealed that in comparison to controls, the serum level of miRNA-133 in breast cancer was statistically significantly lower. Thus, providing credence to the theory that miRNA-133 functions as a tumor

suppressor gene influencing the onset and spread of breast cancer which is consistent with results of other studies conducted globally (21,22).

According to the current study's findings, miRNA-133 was significantly downregulated in breast cancer patients' serum compared to normal control serum. This finding aligns with the findings of Jang JY et al., who reported that miRNA-133 was downregulated in patients' serum compared to normal control serum, demonstrating that miRNA-133 is thought to be a tumor suppressor gene in breast cancer (23). Further, the results of the study revealed that another non-coding RNA, miRNA-182, was statistically significantly higher in breast cancer serum as compared to the serum of healthy controls which is similar to a study conducted by Hagrass et al., which also revealed that breast cancer patients' serum had higher levels of miRNA-182 as compared to the serum of healthy controls (24). The results obtained in the present study indicated that miRNA-182 was highly expressed as well as that lymph nodes were involved similarly Hagrass et al., discovered that serum miRNA-182 expression was significantly higher in breast cancer patients with lymph node involvement (24). According to the American Society of Clinical Oncology and the National Comprehensive Cancer Network (NCCN) guidelines they prevent the use of serum CA-15.3 and CEA for therapeutic response monitoring, routine surveillance, or breast cancer screening and treatment direction (25). The strength of this study is that allows the comparison of miRNA expression between groups and helps in identifying associations between breast cancer. The main limitation of this study is the study design which introduces recall bias and establishes causation which remains a challenge.

Conclusions

Serum miRNA-182 and miR-133 have the potential to be used as minimally invasive biomarkers for breast cancer diagnosis because miRNAs can exist steadily in circulating blood which are simple to quantify.

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