RESEARCH ARTICLE

Editorial Process: Submission:03/22/2025 Acceptance:07/14/2025

Role of Circulating *ARID1A* mRNA as a Potential Biomarker in Female Breast Cancer Patients

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Abstract

Background: Early diagnosis of breast cancer is crucial for successful management and better disease outcomes. Using blood samples as a source of minimally invasive cancer biomarkers, is an essential research area. We aimed to evaluate the role of serum *ARID1A* mRNA as a circulating biomarker in breast cancer patients. **Methods:** This case control study enrolled 60 newly diagnosed female breast cancer patients and 60 age matched control subjects. Total RNA extraction was performed from serum samples of all subjects, followed by reverse transcription and real time polymerase chain reaction for *ARID1A* gene expression. The expression level was studied for its correlation with different clinical and histopathological features of the study patients. **Results:** Serum level of *ARID1A* mRNA was significantly lower in breast cancer patients than in control subjects ($p<0.001$). Median (min.-max.) of serum *ARID1A* in patients was 0.27 (0.005 – 3.92), versus 0.99 (0.27 – 3.53) in control subjects. Using ROC curve, *ARID1A* mRNA showed an AUC of 0.898, and 88.33% diagnostic sensitivity, 81.67% specificity. Low expression was significantly associated with increased tumor size, estrogen and progesterone receptor negativity, high Ki-67 labelling index, and high histological grade. No significant association was detected with HER2 status, tumor histopathological type, or lymph node spread. **Conclusion:** *ARID1A* mRNA in serum is a promising circulating biomarker that can help in diagnosis of breast cancer. Furthermore, the presented correlations indicate a potential prognostic value of serum *ARID1A* mRNA, where decreased level of this biomarker was associated with histopathological findings suggestive of a poor outcome.

Keywords: ARID1A- breast cancer- gene expression- biomarker- real-time PCR.

Asian Pac J Cancer Prev, **26 (7)**, 2679-2686

Introduction

Breast cancer represents the most common type of malignant tumors in women [1]. Several factors play important roles in breast cancer tumorigenesis. These mainly include hormonal factors, family history, genetic factors, dietary habits, alcohol consumption and obesity [2]. Despite the great advance in diagnosis and targeted treatment of this type of malignancy, the desired improvement in morbidity and mortality is still not achieved [3]. Earlier diagnosis gives a greater chance of survival, and the disease prognosis is greatly related to the stage at which disease is diagnosed. Lumpectomy, hormone therapy and other minimally invasive treatment options are given to patients with a favourable prognosis. While patients with a poor prognosis are treated using more extensive surgical excision and one or more chemotherapy medications [4].

Currently, imaging techniques, mainly mammography, are used to screen patients for breast cancer, although they have several limitations such as elevated cost and low sensitivity [5]. Histopathological assessment of a suspected tissue is still the most reliable procedure to confirm a diagnosis of breast cancer [6]. Therefore, many research efforts have been focused on the development of reliable non-invasive biomarkers that may help earlier diagnosis of breast cancer [7].

Adenine thymine rich interactive domain-containing protein 1A (*ARID1A*) gene, is a newly discovered tumor suppressor gene which encodes the BRG1-associated factor 250a (BAF250a) protein [8, 9]. The encoded protein is an important member of the SWI/SNF chromatin remodelling complex. Chromatin remodelling is a dynamic process that gives an access to the underlying DNA in nucleosomes in order to facilitate activation or repression of gene transcription. This process involves many histone modifying enzymes and ATP dependent remodelling complexes. One important member of these complexes is the SWI/SNF complex which is composed of more than 15 subunits. BAF250a protein is the main non catalytic subunit of SWI/SNF complex. This subunit binds to target DNA, guides the remodelling complex

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and directs the ATPase activity. Besides, C- terminal end of the BAF250a protein stimulates activation of glucocorticoid receptor- dependent transcription factors. Mutations of *ARID1A* gene have been shown to affect the expression of multiple genes (including cyclin-dependent kinase inhibitor *CDKN1A, SMAD* family member 3, mutL homolog 1 *MLH1* and phosphatidylinositol kinase *PIK3IP1*) through chromatin remodelling dysfunction. These alterations lead to dysregulation of cell cycle, cell proliferation, apoptosis and DNA repair with subsequent carcinogenesis [10, 11]. Alterations in the expression level of *ARID1A* gene have been correlated with prognosis in multiple types of cancer [10-13]. In breast cancer, recent studies have mainly shown that mutations of this gene and downregulation of the encoded protein in breast cancer tissue specimens, are linked to tumor aggressiveness and decreased patients' survival [13]. Therefore, altered expression of *ARID1A* gene, which could be detected by molecular or immunohistochemical methods on tissue samples, may be a prognostic factor in breast cancer. In addition, it may be used in the future as a molecular target in the targeted treatment plan of breast cancer [2]. However, to the best of our knowledge, the levels of *ARID1A* mRNA in peripheral blood of breast cancer patients have not been studied yet.

The aim of the current work was to study the expression level of *ARID1A* gene in peripheral blood samples of female breast cancer patients as a minimally invasive biomarker in these patients, and to correlate it with the clinical and pathological characteristics of the study patients.

Materials and Methods

Ethical approval

Before starting this research work, the study obtained approval of the Ethics Committee of Alexandria University, Faculty of Medicine (IRB 00012098, FWA 00018699, serial number 0306521). All patients and control subjects gave written informed consent before participation in the study. The study was carried out following the Declaration of Helsinki guidelines.

Sample size estimation

Sample size was calculated using Power Analysis and Sample Size Software (PASS 2020) "NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass". We found that a minimal total hypothesized sample size of 120 eligible subjects (60 female breast cancer cases vs. 60 matched control subjects), is needed to study the expression level of *ARID1A* gene in peripheral blood samples of female breast cancer patients as a minimally invasive biomarker in these patients, and to correlate it with the clinical and pathological characteristics of the study patients; taking into consideration a 95% confidence level, an effect size of 22% and 80% power, using Chi-square test [14, 15].

Study subjects and study design

The present work is a case-control study including two groups: female patients with a new diagnosis of breast cancer (cases) and healthy female subjects (controls). The enrolled patients visited the oncology clinic of Alexandria Main University Hospital in the period from February 2024 to January 2025. Any patient who had undergone surgery for breast cancer, patients who began neoadjuvant treatment, breast cancer recurrent cases, patients having another cancer type, patients with autoimmune diseases, cases having a chronic liver disease or a chronic lung disease, and patients having incomplete data, were excluded. Applying these criteria, 60 female breast cancer patients were included in this research, and as a control group, 60 healthy female subjects, matched in age, were enrolled. Precise clinical examination and negative breast imaging results were used to rule out breast cancer in the control group.

All included breast cancer patients had detailed history assessment and full clinical examination. Radiological assessments including mainly ultrasound (US) on both breasts, mammogram and other imaging modalities to look for metastasis, were done. Diagnosing breast cancer in patients relied on histopathological examination of US guided core biopsy, followed by immunohistochemistry (IHC) for hormonal receptor and Human Epidermal Growth Factor Receptor 2 (HER2) status assessment. All relevant information of enrolled patients were gathered from their medical records. The TNM (Tumor, Node, Metastasis) system was used for clinical staging of the study patients.

Molecular analysis

Three mL venous blood samples were collected from all patients and control subjects, on plain vacutainer tubes, before starting any treatment (surgery or neoadjuvant treatment). Samples were kept at room temperature for twenty min to clot, then centrifuged at 4000 rpm for ten min. Separated serum was stored frozen at -80º C before extraction.

Total RNA was extracted from serum samples according to manufacturer's instructions, using miRNeasy Kit (Qiagen, Germany) Cat no. 217004. Briefly, 5 volumes Qiazol Lysis Reagent were added to one volume of serum, well mixed and kept at room temperature (15–25°C) for 5 min. Then, chloroform was added and incubated at 15–25°C for 3 min. After centrifugation, the upper aqueous part was pipetted into another tube, followed by adding absolute ethanol and good mixing. The sample was then pipetted into a mini spin column (that was put in a new collection tube), centrifuged and the flow-through was discarded. To remove any impurities, two washing steps were performed. To dry the membrane, spin column was put into a new collection tube, and centrifuged at full speed for 5 min. Elution was done by pipetting 25 µL RNase-free water onto the spin column membrane, and centrifugation for 1 min at full speed. Extracted RNA purity and concentration were checked using Nano Drop 2000 Spectrophotometer (Thermo Scientific, USA) and samples were then stored at -80º C till further use.

For the synthesis of complementary DNA (cDNA), we used TOP script™ RT Dry MIX kit (Enzynomics, Korea), CAT number RT220. Purified RNA was mixed with the master mix for reverse transcription, and completed

to a total volume of 20 µL. Reverse transcription was performed on the thermal cycler SimpliAmp (Applied biosystems, Singapore) that was set up as follows: 10 min at 25°C, followed by 60 min at 50°C, then 5 min at 95°C. After that, tubes containing cDNA were kept at -20ºC before subsequent use in PCR.

Quantitative real-time PCR (qPCR) of the *ARID1A* gene and the house keeping gene (*GAPDH*) were done on QIAGEN Rotor-GeneQ (Germany). We used the TOPreal™ qPCR 2X SYBR Green kit, from Enzynomics, Korea (cat no. RT500S). Each amplification mix consisted of: 10 µL SYBR Green mix, 20 ng of cDNA, one µL of each primer (forward and reverse) at a concentration of 10 pmol/µL, and RNase free water to reach a reaction volume of 20 µL. PCR tubes were incubated at 95°C for fifteen minutes (Taq enzyme activation). Then, 45 cycles of: 95°C for ten seconds (denaturation), 57°C for fifteen seconds (annealing), 72°C for thirty seconds (extension). We utilised the Primer-Blast tool to verify the applied primer sequences, (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). For relative quantification of serum *ARID1A* mRNA level, we used comparative CT method ($2^{-\Delta\Delta CT}$) [16]. The supplementary file S1 shows the primer sequences that were used in PCR.

Statistical analysis

The analysis was performed employing IBM SPSS software (package version 20.0). Qualitative parameters were expressed as numbers and percentages. Kolmogorov-Smirnov test was used to test normality of included quantitative data that were represented as mean, standard deviation, median and inter-quartile range. In order to compare quantitative data between two groups, Student t-test was applied for normally distributed variables, while Mann Whitney test was applied for non-parametric quantitative variables. We used Spearman coefficient for testing correlation of *ARID1A* data with quantitative parameters. Mann Whitney test was utilised to assess correlation for two groups and Kruskal Wallis test was employed for more than two groups. For assessing the diagnostic performance of *ARID1A* mRNA, Receiver Operating Characteristic curve (ROC curve) was used, with area under curve about 100% denoting optimal performance for the test. When p-value was less than 0.05, the obtained results were defined significant.

Results

Baseline features of the study subjects

The present study included 120 subjects who were divided into two groups: sixty female patients with a first diagnosis of breast cancer (cases) and sixty control subjects. The mean age was 49.42 ± 8.94 years in control participants while it was 50.10 ± 9.37 in the included patients. Median body mass index, BMI (IQR), was 28.0 (26.0 – 29.0) % in patients, and 28.5 (26.0 – 30.0) % in control group. Fifty percent of cases were postmenopausal and 55% were postmenopausal in control group. There was no significant difference between both groups regarding these parameters, and no significant difference was observed with regard to coexisting morbidities encountered in the enrolled subjects, Table 1.

The clinical and histopathological features of patients are described in Table 2. According to clinical TNM staging, 16.7 % of patients presented in stage I, 65% presented in stage II and 18.3 % presented in stage III. Breast US and mammography showed that 31.7% of patients were BIRADS 4 (Breast-Imaging Reporting and Data System), 68.3 % were BIRADS 5. Core biopsy histopathological examination showed that main histopathological type was invasive ductal carcinoma (IDC; 88.3%) and the main histological grade was grade 2 (65%). Results of IHC showed that 83.3 % of cases had estrogen receptor (ER) positivity, 70 % had

Table 1. Comparison between Patients and Controls according to According to Demographic Data and Associated Comorbidities

Parameter	Patients (n = 60)	Control (n = 60)	Test of Significance	p
Age (years)				
Min. – Max.	30.0 – 73.0	32.0 – 66.0	t= 0.409	0.684
Mean ± SD.	50.10 ± 9.37	49.42 ± 8.94		
Median (IQR)	48.0 (43.0 – 58.0)	49.5 (43.0 – 55.0)		
BMI (kg/m^2)				
Min. – Max.	20.0 – 33.0	22.0 – 33.0	U= 1492	0.103
Mean ± SD.	27.27 ± 2.85	28.17 ± 2.64		
Median (IQR)	28.0 (26.0 – 29.0)	28.50 (26.0 – 30.0)		
Menopause				
No	30 (50.0%)	27 (45.0%)	χ^2= 0.301	0.583
Yes	30 (50.0%)	33 (55.0%)		
Comorbidities				
DM (diabetes mellitus)	27 (45.0%)	24 (40.0%)	χ^2=0.307	0.58
HTN (hypertension)	21 (35.0%)	19 (31.7%)	χ^2=0.150	0.699
IHD (ischemic heart disease)	12 (20.0%)	13 (21.7%)	χ^2=0.051	0.822

IQR, Inter quartile range; SD, Standard deviation; U, Mann Whitney test; χ^2, Chi square test; t, Student t-test ; p, p-value for comparing between the two studied groups

Table 2. Distribution of the Studied Patients According to Clinical and Pathological Parameters

Parameter	No. (%)
Tumor size (cm)	
T1 (≤2)	12 (20.0%)
T2 (2 – ≤5)	33 (55.0%)
T3 (>5)	15 (25.0%)
LN spread	
Negative	41 (68.3%)
Positive	19 (31.7%)
Metastasis	
Negative	60 (100.0%)
Positive	0 (0.0%)
Mammogram	
BIRADS 4	19 (31.7%)
BIRADS 5	41 (68.3%)
Histopathological type	
IDC	53 (88.3%)
ILC	7 (11.7%)
Grade	
I	7 (11.7%)
II	39 (65.0%)
III	14 (23.3%)
ER status	
Negative	10 (16.7%)
Positive	50 (83.3%)
PR status	
Negative	18 (30.0%)
Positive	42 (70.0%)
HER2 status	
Negative	42 (70.0%)
Positive	18 (30.0%)
Molecular sub type	
Luminal A	3 (5.0%)
Luminal B, HER2 negative	34 (56.7%)
Luminal B, HER2 positive	13 (21.7%)
Triple negative	5 (8.3%)
HER2 overexpression	5 (8.3%)
Stage	
I	10 (16.7%)
II	39 (65%)
III	11 (18.3%)

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; PR, progesterone receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; BIRADS, breast-imaging reporting and data system

progesterone receptor (PR) positivity, and 30 % showed *HER2* positivity. The Ki-67 index ranged from 11 – 75 %, with a mean of 42.55 ± 17.15 % and a median of 41.5 %. The main molecular subtype was luminal B, *HER2* negative (56.7 %).

Recruited breast cancer patients were treated based on clinical TNM staging at presentation and their IHC results. Accordingly, 51.7% of patients had upfront surgery followed by adjuvant treatment. The remaining 48.3% of patients started with neoadjuvant treatment (chemotherapy, hormonal treatment, targeted therapy in cases with *HER2* positivity), followed by surgery.

ARID1A expression and correlation with patients' features

The expression level of the studied gene (ARID1A), in serum of all study subjects, is illustrated in Table 3 and Figure 1. In the patients' group, median of serum *ARID1A* mRNA (min.-max.) was 0.27 (0.005 – 3.92), while in the control group, it was 0.99 (0.27 – 3.53).

ARID1A mRNA level was significantly lower in cancer patients in comparison to control subjects ($p<0.001$). We used logistic regression multivariate analysis to adjust for demographic parameters and associated benign diseases in the study participants. Downregulation of *ARID1A* mRNA was found as a significant independent discriminator between breast cancer patients and control subjects, after adjusting for age, BMI, menopausal state, presence of DM, HTN, and IHD. This is illustrated in the supplementary file S2, where in univariate analysis, *ARID1A* mRNA had an Odds ratio of 0.071, with a Confidence Interval (C.I: 0.025 – 0.202), and a p-value <0.001. Additionally, in the multivariate analysis, *ARID1A* mRNA had an Odds ratio of 0.066, C.I (0.022 – 0.204), and a p-value <0.001.

Moreover, we observed a statistically significant correlation between the low *ARID1A* mRNA and the increase in tumor size, advanced histological grade, and increased Ki-67 index, with p - value <0.001 for each parameter. It was also significantly associated with ER negativity and PR negativity, ($p<0.001$), as shown in Figure 2. Serum *ARID1A* gene expression was significantly lower in stages II, and stage III patients than in stage I patients. The expression was significantly lower in triple negative and *HER2* enriched molecular subtypes compared to the expression level in luminal subtypes.

Other clinical and pathological parameters didn't show statistically significant correlations with *ARID1A* mRNA level (Table 4). Using ROC curve, we studied the ability of serum *ARID1A* mRNA to differentiate between breast cancer and control subjects, Figure 3. The best cut off value for diagnosing breast cancer was ≤0.651, with an AUC of 0.898, showing 88.33 % diagnostic sensitivity and 81.67 % specificity, 82.8 % positive predictive value and 87.5

Table 3. Comparison between Patients and Controls according to According to *ARID1A* Expression

ARID1A expression	Patients (n = 60)	Control group (n = 60)	U	p
Min. – Max.	0.005 – 3.92	0.27 – 3.53	367.00*	<0.001*
Median (IQR)	0.27 (0.12 – 0.47)	0.99 (0.74 – 1.45)		

IQR, Inter quartile range; U, Mann Whitney test; SD,standard deviation; p, p value for comparing between the two studied groups (patients and controls); *, Statistically significant at $p \leq 0.05$

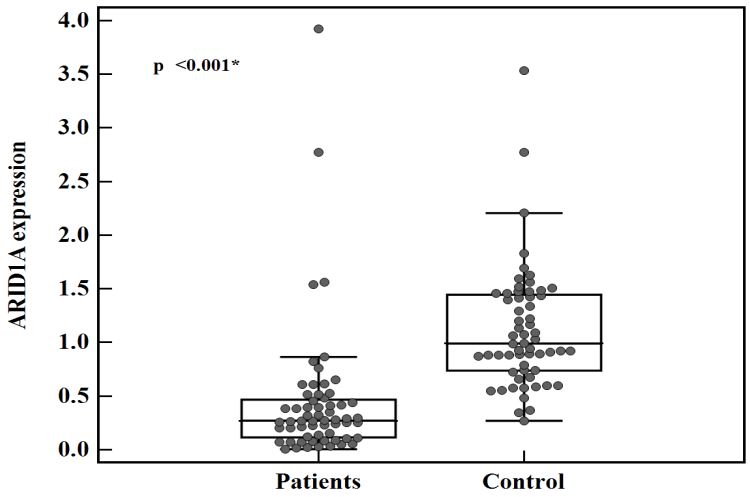

Figure 1. Comparison between the Two Studied Groups According to Serum *ARID1A* Expression

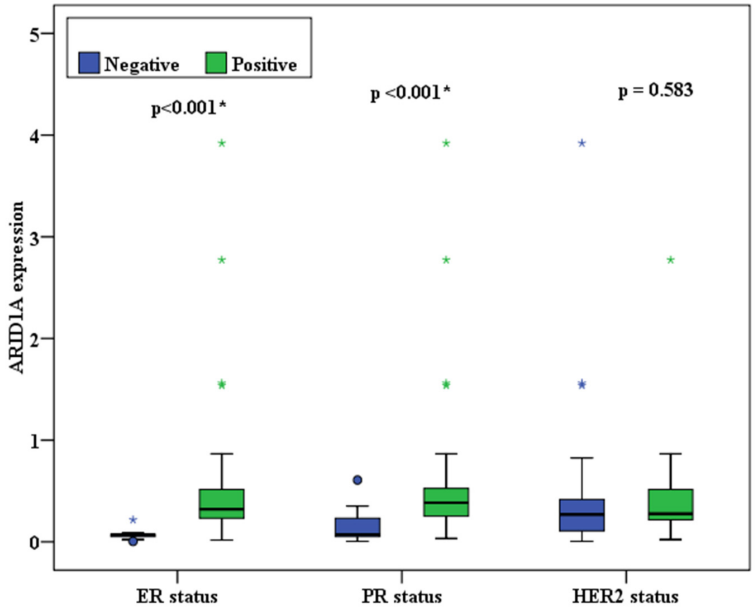

Figure 2. Relation of Serum *ARID1A* Expression with ER Status, PR Status and *HER2* Status in Patients' Group

%negative predictive value.

Discussion

Most of the research work studying *ARID1A* gene expression in breast cancer have reported decreased expression of this gene in malignant breast tissue compared to normal breast tissue specimens. In addition, this was correlated with parameters of clinical deterioration, poor treatment response and shorter survival [2, 13, 15, 17, 18].

Earlier studies investigating *ARID1A* in breast cancer have utilized excised (post-mastectomy) malignant breast tissue samples in comparison to normal breast tissue samples. Peripheral blood samples are easier to obtain and many studies have focused on finding beneficial non-invasive and minimally invasive biomarkers in different types of cancer [19-22]. Gao X et al. has investigated *ARID1A* mRNA as a serum biomarker in gastric cancer

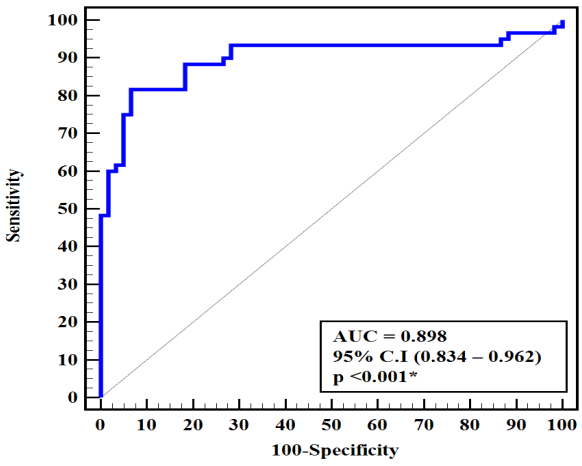

Figure 3. ROC Curve for Serum *ARID1A* Expression to Discriminate Breast Cancer Patients from Control Subjects

Table 4. Relation between *ARID1A* mRNA and Different Parameters of the Included Patients

Parameter	No.	*ARID1A* mRNA		Test of significance	p
		Mean ± SD.	Median (Min. – Max.)		
Menopause					
No	30	0.48 ± 0.57	0.32 (0.005 – 2.77)	U= 366	0.214
Yes	30	0.40 ± 0.70	0.25 (0.017 – 3.92)		
Tumor size (cm)					
T1 (≤2)	12	0.86 ± 0.75	0.57 (0.203 – 2.77)	H= 21.390*	<0.001*
T2 (2 – ≤5)	33	0.42 ± 0.65	0.28 (0.022 – 3.92)		
T3 (>5)	15	0.14 ± 0.18	0.07 (0.005 – 0.61)		
LN spread					
Negative	41	0.45 ± 0.65	0.30 (0.017 – 3.92)	U= 328	0.328
Positive	19	0.41 ± 0.62	0.24 (0.005 – 2.77)		
Mammogram					
BIRADS 4	19	0.60 ± 0.87	0.38 (0.03 – 3.92)	U= 272.5	0.063
BIRADS 5	41	0.36 ± 0.49	0.25 (0.005 – 2.77)		
Histopathological type					
IDC	53	0.42 ± 0.65	0.27 (0.005 – 3.92)	U= 138.5	0.286
ILC	7	0.55 ± 0.52	0.51 (0.104 – 1.56)		
Grade					
I	7	1.26 ± 0.77	0.87 (0.525 – 2.77)	H= 21.728*	<0.001*
II	39	0.39 ± 0.60	0.27 (0.033 – 3.92)		
III	14	0.16 ± 0.18	0.09 (0.005 – 0.51)		
ER status					
Negative	10	0.07 ± 0.06	0.07 (0.005 – 0.22)	U= 33.00*	<0.001*
Positive	50	0.51 ± 0.67	0.32 (0.017 – 3.92)		
PR status					
Negative	18	0.15 ± 0.15	0.07 (0.005 – 0.61)	U= 105.50*	<0.001*
Positive	42	0.56 ± 0.72	0.38 (0.033 – 3.92)		
HER2 status					
Negative	42	0.42 ± 0.65	0.27 (0.005 – 3.92)	U= 344	0.583
Positive	18	0.47 ± 0.62	0.28 (0.022 – 2.77)		
Molecular sub type					
Luminal A	3	2.34 ± 1.37	1.56[a] (1.538 – 3.92)	H= 27.582*	<0.001*
Luminal B, HER2 negative	34	0.31 ± 0.19	0.28[b] (0.017 – 0.82)		
Luminal B, HER2 positive	13	0.62 ± 0.68	0.44[ab] (0.232 – 2.77)		
Triple negative	5	0.05 ± 0.03	0.06[c] (0.005 – 0.07)		
HER2 overexpression	5	0.09 ± 0.07	0.07[c] (0.022 – 0.22)		
Stage					
I	10	0.73 ± 0.46	0.57[a] (0.232 – 1.56)	H= 13.625*	0.001*
II	39	0.42 ± 0.72	0.27[b] (0.02 – 3.92)		
III	11	0.22 ± 0.27	0.11[b] (0.005 – 0.87)		

SD, Standard deviation; U, Mann Whitney test; H, for Kruskal Wallis test; p, p value for relation between ARID1A expression and different parameters, *: Statistically significant at p ≤0.o5 ; Medians with totally Different letters (a-c) are significantly different; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; *HER2*, human epidermal growth factor receptor 2; BIRADS, breast-imaging reporting and data system

patients and reported significantly decreased levels of this circulating molecular marker in serum samples of gastric cancer patients [23]. To the best of our knowledge, the current work is the first one to study the circulating level of *ARID1A* mRNA in peripheral blood samples of breast cancer patients. Our results are generally comparable with the results of previous work performed on breast tissue specimens. We observed decreased *ARID1A* mRNA level in the serum samples of breast cancer patients compared to matched serum samples from healthy control subjects.

Additionally, using ROC curve in our study, *ARID1A* mRNA had 88.33% diagnostic sensitivity and 81.67 % specificity. These findings indicate a potential role of serum *ARID1A* mRNA to help in the diagnosis of breast cancer, and are consistent with the previously reported tumor suppressor function of *ARID1A* gene [11, 24, 25].

Our findings are in agreement with the results reported by Uncle et al. in their study. They investigated *ARID1A* gene expression in breast cancer surgical specimens using immunohistochemistry technique, and they found loss of expression in 42% of cases. In addition, the study showed a significant correlation of the loss of *ARID1A* gene expression with both estrogen receptor negativity and progesterone receptor negativity. However, they found a significant correlation with lymph node spread, that was not significant in our results. In their study, the low expression was also associated with the presence of in situ component [2].

Cho HD et al. also showed decreased *ARID1A* expression in 71% of breast cancer tissue specimens, using immunohistochemistry on tissue microarrays. When comparing the correlation results of their study to our findings, some discrepancies were found. Their results showed there was no significant correlation with estrogen receptor or progesterone receptor expression. Significant correlations were detected with the presence of lymph node spread, low Ki-67, and negative p53 expression. The correlation with histopathological grade was not statistically significant (p= 0.056). However, Cho HD et al. also suggested poor prognostic value of low *ARID1A* mRNA in breast cancer patients, by reporting a significant correlation of low *ARID1A* expression level with shorter patient survival [18].

Our results were in accordance with Zhang X et al. who reported low level of *ARID1A* mRNA in 55% of breast cancer tissue specimens in comparison with normal matched breast tissue specimens. The low gene expression was associated with larger tumor size, advanced stage, high Ki-67 labelling index and with estrogen receptor negativity. All of these correlations were found similarly in our study. However, different from our results, Zhang X et al. found no significant correlation with progesterone receptor status, and they found a significant association with the presence of lymph node metastasis. They also reported an association between the low expression level and triple negative molecular subtype. In their study, Zhang X et al. added that higher BAF 250a protein expression (encoded by *ARID1A* gene) was associated with higher five-year survival rate, which is also in agreement with the tumor suppressor function of the *ARID1A* gene [15].

Zhao J et al. studied the expression of the *ARID1A* encoded BAF250a protein in breast cancer tissue, and its association with the clinicopathological characteristics of the patients. Their findings were in line with the reported function of *ARID1A* gene. The protein expression was significantly lower in breast cancer specimens than in matched normal breast tissue. They reported a significant association between the low protein expression and the increase in tumor size, presence of lymph node spread, advanced malignancy stage, higher histopathological grade, estrogen receptor and progesterone receptor negative status and poor survival outcome [13].

It is obvious that downregulation of *ARID1A* in breast cancer tissue, in previous research work, was found to be associated with parameters suggesting possible worse disease outcomes, including mainly estrogen receptor negative status and advanced tumor stage. Interestingly, our study has yielded similar findings using serum samples from the included breast cancer patients. The observation of some discrepancies in the correlation results between the current study and previous studies (e.g PR status and lymph node involvement), could be attributed to factors such as difference in the sample type, differences in the study sample size and variation in the ethnic background of the included population in each study.

Limitations

The study patients did not include any case of breast cancer with positive metastasis, which represented a limitation of the current work. Other limitations included relatively modest sample size, non-availability of p53 expression results, and the lack of correlation with treatment response because of inability to follow-up all included patients. In addition, lack of patient follow-up led to the absence of survival analysis and inability to correlate *ARID1A* expression results with survival outcomes of the patients.

Conclusion

The present study showed that serum level of *ARID1A* mRNA was significantly lower in breast cancer patients in comparison with normal subjects, indicating a promising diagnostic role of this molecular marker in breast cancer. Moreover, the decreased level was significantly associated with increased tumor size, estrogen and progesterone receptor negativity, higher Ki-67 index, and higher tumor grade. The presented correlations suggest a potential poor prognostic value of low serum *ARID1A* mRNA in breast cancer patients. To further support these results, we recommend the investigation of serum *ARID1A* expression in female breast cancer through future multi-center studies, recruiting more cases with different breast cancer stages and different molecular subtypes. Further studies measuring circulating *ARID1A* mRNA together with measurement of the encoded protein, in female breast cancer patients, are also recommended.

Author Contribution Statement

E.M.A has developed the study concept and design. All authors contributed to the practical part of the study: M.A.A and I.I.A performed sample collection and gathering of patients data, E.M.A and E.S.N performed laboratory molecular testing and patients data analysis. E.M.A. and E.S.N wrote the main manuscript text. All authors revised the manuscript and approved it for publication.

Acknowledgements

We want to acknowledge the Molecular Biology

Unit at the Medical Research Centre (MRC), Faculty of Medicine, Alexandria University, for providing instrument facilities to our study.

Data availability

All data which support the current research findings are available within this paper and its supplementary files.

Ethical approval

Before starting this research work, the study obtained approval of the Ethics Committee of Alexandria University, Faculty of Medicine (IRB 00012098, FWA 00018699, serial number 0306521). The study was carried out following the Declaration of Helsinki guidelines.

Informed consent

All patients and control subjects gave written informed consents before participation in the study.

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