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Genotypic and Phenotypic Study of *PDCD4* gene Concerning *micro RNA-21* and *micro RNA-449b* Polymorphism in Breast Cancer

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Abstract

Breast cancer is a heterogeneous disease with a significant impact of genes involved in apoptosis. Hence, this study aims to assess the contribution of programmed cell death protein 4(PDCD4) miR-21, miR-449b and *PDCD4* genes polymorphism in breast cancer pathogenicity. A total of 180 blood samples were collected from Iraqi women aged between 15-80 years, 120 samples were included from breast cancer patients (divided into two groups of 60 patients under treatment and 60 post treatments and recovery) then 60 healthy women as a control group. The extracted genomic DNA was amplified by PCR technique for the loci of interest and PCR products of the tested samples were genotyped using TaqMan real time fluorescent oligonucleotide for SNPs analysis. PDCD4 protein concentration in serum was estimated utilizing ELISA**.** The results showed significant association (P = 0.016) of miR-449b rs10061133:A>G polymorphism (AA, AG and GG) on the PDCD4 serum levels (3.62 ng/L, 4.75 ng/L and 2.58 ng/L respectively). PDCD4 serum showed to differ significantly (p = 0.001) among the investigated groups: treatment (5.39 ng/L), post-treatment (3.42ng/L), and control (3.19 ng/L). An evaluation of the effects of breast cancer risk factors on the studied parameter revealed that breastfeeding appears to significantly influence PDCD4 serum levels. Women who breastfed exhibited a mean PDCD4 concentration of 4.40 ng/L, compared to 3.25 ng/L for those who did not breastfeed (P=0.026). Additionally, the AUC value (0.7) suggests that PDCD4 protein could be a good predictive marker for treatment response. Overall, the phenotypic expression of PDCD4 demonstrated to be significantly influenced by polymorphisim of PDCD4, *miR-21* and *miR-449b* SNPs, with a considerable impact of breastfeeding in increasing PDCD4 levels that could be utilized **to** promote breast cancer prevention strategies.

Keywords: breast cancer, breastfeeding, polymorphism, PDCD4, micro RNA

دراسة النمط الوراثي والمظهري للجين *PDCD4* وعلاقته بتعدد الأشكال للجينات *micro RNA-21* و *micro RNA-449b* في سرطان الثدي

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الخلاصة

سرطان الثدي هو مرض مختلف المصادر التي تؤثر على تعطيل الجينات المشاركة في موت الخلايا المبرمج. تهدف الدراسة إلى تقييم بروتين موت الخلايا المبرمج 4 (PDCD4) وعلاقته بتعدد الأشكال الوراثية لجينات *miR-21* و*miR-449b* و*PDCD4* في سرطان الثدي. تم جمع 180 عينة دم من النساء العراقيات الذين تتراوح أعمارهم بين 15-80 سنة، بضمنها 120 عينة من مريضات سرطان الثدي (مقسمة إلى مجموعتين من 60 مريضة تحت العلاج و 60 بعد العلاج والشفاء) و 60 امرأة سليمة كمجموعة سيطرة . تم استخلاص الحمض النووي الجينومي DNA وتضخيم PCR للجينات المدروسة، تم التنميط الجيني لجينات العينات المدروسة باستخدام تقنية TagMan real time لتحليل SNPs. بالإضافة إلى ذلك، تم تقدير مستويات بروتين PDCD4 في المصل باستخدام ELISA. ظهر تاثير معنوي (p=0.016) لتعدد اشكال جين rs10061133 *miR-449b* على تركيز بروتين PDCD4 المصلي حيث كانت التراكيز في الطرز الوراثية AA (3.62) و AG (4.75) و GG (2.58) نانوغرام/ لتر. كما أظهرت تراكيز بروتين PDCD4 المصلي اختلافًا كبيرًا (p<0.001) بين المجموعات المدروسة: حيث كان تركيزه في مجموعة المرضى تحت العلاج (3.19 نانوغرام / لتر)، ومرضى ما بعد العلاج (5.39 نانوغرام / لتر)، ومجموعة السيطرة (3.42 نانوغرام / لتر). بالإضافة إلى ذلك، يبدو أن الرضاعة الطبيعية تؤثر بشكل معنوي (P = 0.026) على تركيز PDCD4 في المصل حيث كان تركيزه (4.40 نانوغرام / لتر لمجموعة الرضاعة الطبيعية مقابل 3.25 نانوغرام / لتر للمجموعة غير المرضعة). كانت قيمة AUC (0.7) لبروتين PDCD4 مؤشرًا جيدًا يستخدم لتقييم الاستجابة للعلاج. بشكل عام، أثبت التعبير المظهري (تركيز PDCD4) أنه يتأثر بشكل كبير بتعدد الاشكال جينات ال *PDCD4* و*miR-21* و *miR-449b*. ظهرتأثير كبير للرضاعة الطبيعية في زيادة PDCD4 التي يمكن استخدامها لتعزيز استراتيجيات الوقاية من سرطان الثدي.

Introduction

Breast cancer is a highly diverse disease with various subtypes, each of which responds differently to treatment [1]. In Iraq, breast cancer accounts for approximately 34% of cancer cases among women [2]. Given the wide range of cellular and molecular differences within breast cancer, it is essential to analyze multiple genetic changes simultaneously to comprehend their impact on the disease's development and progression[3]. For the past three decades, a fundamental objective in clinical oncology has been to create therapies that effectively eliminate cancer cells by promoting apoptosis, a process of programmed cell death. This apoptosis process is initiated by many factors such as cellular stress, DNA destruction, and immune investigation, which activate multiple signaling pathways. The communication between apoptosis pathways and other signaling mechanisms can also affect cell death outcomes. What's notable is that the successful interpretation of pro-apoptotic treatments into clinical practice needs a combination of drug discovery research and more understanding of cancer biology. While therapy can lead to the death of cancer cells, growth, and spread of resistant cells can eventually prove deadly[4].

The programmed cell death 4 (*PDCD4*) gene acts as a cancer suppressor and shows a title role in several cellular functions such as apoptosis, transcription, and regulation of signal transduction pathways. PDCD4 is involved in regulating programmed cell death and also impacts transcription as well as numerous cells signaling pathways through its suppressive effects. The PDCD4 is often downregulated or absent in various types of tumer including breast cancer. Expression of PDCD4 in specific cell types is organized through various pathways. when PDCD4 is reduced in tumor cells, it has been linked to drug resistance, while its presence makes cells more responsive to chemo-radiotherapy [5]. The human *PDCD4* gene was initially identified as a nuclear antigen gene and is located on chromosome 10q25.2, comprising 13 exons [6]. PDCD4's roles can be categorized into two main functions: its

involvement in tumor suppression and its participation in the inflammatory process. Changes in PDCD4 expression are pivotal in cancer development[7]. Increased PDCD4 levels promote apoptosis or cell cycle arrest, inhibit cancer cell invasion, proliferation, and migration, and heighten sensitivity to anticancer drugs. Conversely, reducing PDCD4 expression encourages the invasion and migration of cancer cells[6]. Consequently, irregular PDCD4 expression levels are associated with cancer advancement. Understanding the mechanisms that govern PDCD4 expression and targeting its balance could be beneficial for related treatments. Therefore, therapeutic strategies that focus on manipulating PDCD4 expression hold promise for addressing cancer and inflammatory disorders [8].

The PDCD4 protein is remarkably similar across various species, showing a high degree of conservation [9]. It comprises a 469-amino acid peptide and contains two well-preserved alpha helical MA3 domains. Interestingly, these domains are also found in eukaryotic translation initiation factors, specifically eIF4G I and eIF4G II. Scientific studies have shown that PDCD4 interacts with eukaryotic translation initiation factor 4A (eIF4A) through its MA-3 domain, resulting in bound ribosomal recruitment and protein synthesis In particular, studies have shown that PDCD4 through its MA-3 binding motif upon binding to eIF4A, and thus ribosomes -Inhibit eIF4A RNA helicase activity required for assembly and translation initiation This interaction between PDCD4 and eIF4A restricts mRNA uptake into the ribosome, blocking protein a they are performed at the limits of the semantic level. This inhibition of protein synthesis is a crucial factor in PDCD4's capacity to suppress malignant behaviors[10].

Numerous pathological conditions, including the development of tumors, can be attributed to the misregulation of MicroRNA (miRNA) [11]. In cancer, the expression of many miRNAs is frequently altered, and these changes are closely associated with processes such as tumor formation, invasion, metastasis, and resistance to drugs. There are differences in the miRNA expression between healthy and cancerous tissues[12]. An accumulative evidence recommends that dysregulated miRNAs can role as either oncogenic (promoting cancer) or tumor suppressor factors. [13].

Recent research on *PDCD4* gene has predominantly centered on the role of miRNAs in controlling its expression in tumors. Over 30 miRNAs have been identified as direct inhibitors of PDCD4, and many of these are found to be overexpressed in tumor cells. Furthermore, computational analyses suggest that there may be more than 80 miRNAs with the potential to target PDCD4, underscoring the significant role of miRNAs in regulating PDCD4 expression[14]. These miRNAs specifically interact with the 3' -UTR of PDCD4 mRNA, resulting in its decreased expression. Among these miRNAs, miR-21 has been extensively researched. It is located on chromosome 17q23.2 and is upregulated in various diseases, such as oropharyngeal cancer and salivary adenoid cystic carcinoma [15]. miR-21 has been associated with unfavorable treatment outcomes and reduced survival in multiple types of malignant cancers [16].

Besides miR-21, several other miRNAs have been documented as regulators of PDCD4 expression in different cancer types. For example, miR-181 upregulation is linked to the reduction of PDCD4 expression, which, in turn, facilitates cell proliferation, metastasis, and hampers apoptosis. In a similar vein, in the context of cervical cancer, increased levels of miR-150 encourage the *in vitro* proliferation, migration, and invasion of cervical cancer cells by directly targeting PDCD4 expression [17].
Various non-genetic factors can increase the risk of breast cancer. For instance, women who remain childless or have their first child after the age of 30 face a slightly higher overall risk

of developing breast cancer. Conversely, having multiple pregnancies and becoming pregnant at an early age can reduce the risk of breast cancer. It's important to note that the effects of pregnancy on breast cancer risk can vary among different types of breast cancer, and in some cases, it may even increase the risk. Breastfeeding has been proposed as a means to modestly lower the risk of breast cancer, especially if continued for 1.5-2 years. This reduction in risk may be attributed to the fact that breastfeeding decreases a woman's total number of lifetime menstrual cycles [18]. Research indicates that engaging in regular physical activity, particularly among postmenopausal women, may decrease their risk of developing breast cancer [19]. While the exact mechanism is not yet fully understood, it is believed that physical activity can influence factors such as body weight, inflammation, hormones [20], energy balance and disorder in cytokines such as interleukin 6 [21], all of which could contribute to reducing the risk of breast cancer [22]. The current study aims to assess the influence of PDCD4in breast cancer pathogenicity in relationship to its targeted genetic variants.

Materials and Methods

Subjects and Sampling

Between January and March 2023, a total of 180 blood samples were obtained from Iraqi women ages 15 to 80 years old. The samples comprised 120 collected from breast cancer patients, with 60 from those receiving treatment and 60 from post-treatment patients. An additional 60 samples were secured from healthy women serving as a control group. In summary, during the specified three-month period, blood samples were acquired from 180 Iraqi women across different age groups. Of these samples, 120 came from breast cancer patients divided evenly between current treatment and post-treatment, with the remaining 60 from control subjects without the disease.

The age of the recruited breast cancer patients and their apparently healthy counterpart ranged between 15-80 years old. Breast cancer patients had been diagnosed by specialist physicians at Ramadi Teaching Hospital and Oncology Center, Anbar, Iraq. Participants' demographic information and clinicopathological data were obtained from patients' hospital records and a questionnaire-based data collection for the healthy controls. Informed consent was obtained from all participants enrolled in this study and the ethical approval committee (reference number 22 on 30-1-2023)-University of Anbar, college of science has approved this study.

Estimation of programmed cell death protein 4 (PDCD4) serum levels

To determine the PDCD4 levels in the blood serum of breast cancer patients and their healthy counterparts, serum was separated first by refrigerated centrifugation of the collected blood samples at 3000 rpm for 10 min. PDCD4 level then measured utilizing enzyme-linked immune sorbent assay (ELISA) based on supplied kit with Catalog No: YLA4298HU. www.ylbiont.com**).**

Genomic DNA extraction and quantification

Genomic DNA was obtained from frozen blood samples using the procedure outlined in the Wizard® Genomic DNA Purification Kit, CAT. A1120 by Promega Corporation. To assess the integrity of the extracted DNA, the samples were subjected to electrophoresis on a 1% agarose gel. Additionally, a Quantus Fluorometer was employed to measure the concentration of the extracted gDNA, which was done to evaluate the quality of the samples for use in subsequent applications.

Genotyping of miR-21 rs1292037:T>C, miR-449b rs10061133:A>G and PDCD4 rs6585018:G>A

Genotyping of *miR-21*, *miR-449b* and *PDCD4* genes Polymorphism SNPs of interest were amplified using TaqMan fluorescent oligonucleotide primers and probes. The primers and probe sequences utilized in this study are provided in Table 1, and these primers were provided by Macrogen Company in a lyophilized form. To prepare the primers for use, the lyophilized primers were dissolved in nuclease-free water, resulting in a final concentration of 100 pmol/µl as a stock solution. A stock primer solution stored at -20°C was used to generate a functional primer solution for use in PCR. Specifically, 10 µl of the stock primer solution was added to 90 µl of nuclease-free water, thereby diluting the primers to a functional primer solution. This active solution contained 10 pmol/µl of primerase, which was ideal for use in polymer chains.

Table 1: The primers and probes sequences for the studied SNPs.

SNPs	Primers	Probes
rs1292037:T>C	Forward: ATGGAGGGAGGATTTTATGGAGAA	rs1292037P/C: FamAAGCTGCACTGTGGGT
	Reverse: GAAGGTCAAGTAACAGTCATACAGC	rs1292037-P/T: Hex-ACTAAGCTGCATTGTGGGT
rs10061133:A>G	Forward: GAGAATCGGCAGTGACCTGAA	rs10061133-P/G: Fam-AGGTAGGCAGTGTATCGTTAG
	Reverse: GAAGCAAGTGGCAGGGTAGT	rs10061133-P/A: Hex-AGGCAGTGTATTGTTAGC
rs6585018:G>A	Forward: GCCTGTCCGATTCCTCCTC	rs6585018-P/G Fam-CTGGCCGCTGCTT
	Reverse: AGCATGGGATCTCCAGAAAC	rs6585018-P/A Hex-CTGGCCGTTGCTTT
Annealing Temp. (60ºC)		

For the purposes of this analytical study, DNA samples collected from both breast cancer patients and healthy control subjects were genotyped to determine alleles present at specific single nucleotide polymorphisms (SNPs). The RT-PCR reaction mixture total volume was 20 µl composed 10µl of TaqMan master Mix, 1µl of each fluorescence probes, 1µl from each forward and reverse primer (10µM), 2µl of template DNA, and 4µl nuclease-free water. RT-PCR amplification reaction was performed using a programmed thermocycler with one hold cycle of 95°C for 5 minutes, 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds.

Statistical analysis

All the data were analyzed using IBM® SPSS® statistical software (Version 26.0; IBM SPSS, Armonk, NY, USA. ANOVA test was used to assess the effect of genotypes, cancer treatment, breastfeading, contraceptive, family history of breast cancer, BMI, age on PDCD4 concentration. Receiver Operating Characteristic (ROC) curve analysis was employed to evaluate breast cancer detection and therapeutic response prediction. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Effect of PDCD4 (rs6585018:G>A), miR-21 (rs1292037:T>C) and miR-449b (rs10061133:A>G) SNPs on serum PDCD4 protein concentration in study subjects.

Results showed no significant effect of the rs6585018:G>A genotypes on the concentration of PDCD4, where the protein concentrations for subjects with AA, GA and GG genotypes are 3.82 ng/L, 4.27 ng/L and 2.16 ng/L respectively. Similarly, no significant effect of the rs1292037:T>C genotypes on the concentration of serum PDCD4, where the protein

concentrations for patients with CC, CT and TT genotypes were 1.64 ng/L, 4.19 ng/L and 3.88 ng/L, respectively, as shown in Figure 1.

However, significant effect of the rs10061133:A>G genotypes on the concentration of PDCP4(p = 0.016) where the protein concentrations of the genotypes AA, AG and GG were 3.62 ng/L, 4.75 ng/L and 2.58 ng/L, respectively. A least significant difference (LSD) analysis revealed that PDCD4 concentration varied significantly in study participants carrying the heterozygous AG genotype compared to those with the homozygous AA and GG genotypes (P = 0.026 and P = 0.016 respectively), as illustrated in Figure 1. Specifically, the LSD test showed PDCD4 levels differed statistically between subjects with the AG genotype versus those homozygous for either the A or G allele at the genetic location under investigation.

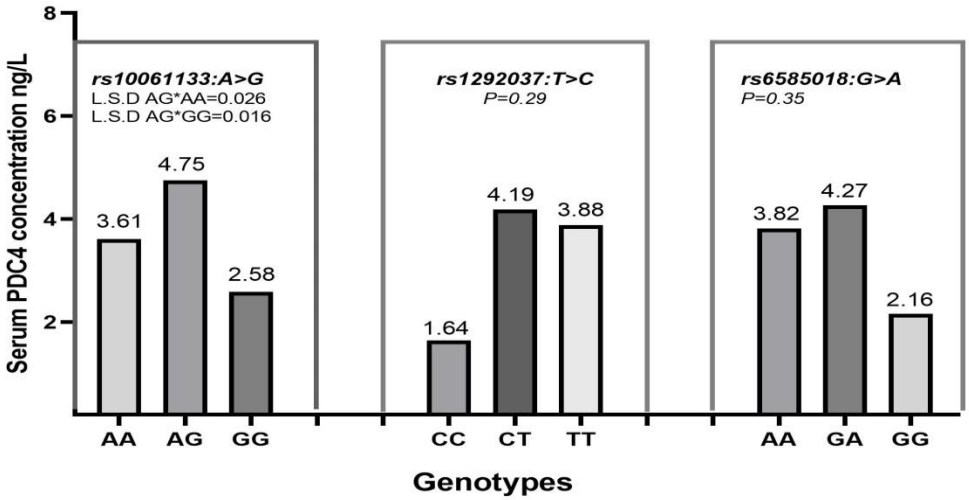

Figure 1: *PDCD4 (*rs6585018:G> A*), miR-21* (rs1292037:T>C) and *miR-449b* (rs10061133:A>G) genes polymorphism in relation to PDCD4 protein concentration in serum in study subjects

According to the findings, individuals with a heterozygous genotype for rs10061133:A>G (totaling 180 subjects) showed higher levels of PDCD4 protein compared to those with a homozygous genotype. This suggests that there is a beneficial effect on fitness related to heterosis resulting from evolutionary processes. Additionally, the heterozygous group displayed lower variation in PDCD4 expression levels attributed to noise-related phenotypic, indicating that this population is more robust when it comes to variations in gene expression due to external factors.

The effect of breast cancer treatment on PDCD4 protein concentration in serum.

The results revealed a notable effect of breast cancer treatment on PDCD4 concentrations. Serum PDCD4 levels were measured in three groups: treatment patients, post-treatment patients, and healthy controls. The mean PDCD4 level was highest among treatment patients at 5.39 ng/L. This was significantly higher than both post-treatment patients and controls. The post-treatment group exhibited a moderately lower PDCD4 concentration of 3.42 ng/L. Meanwhile, the control group without cancer or treatment history had the lowest average PDCD4 level of 3.19 ng/L. Through LSD analysis, it was found that the PDCD4 concentration in the treatment group was significantly higher than control and post-treatment groups, where the significant *P*- values were (P= 0.000 and 0.001, respectively, Figure 2).

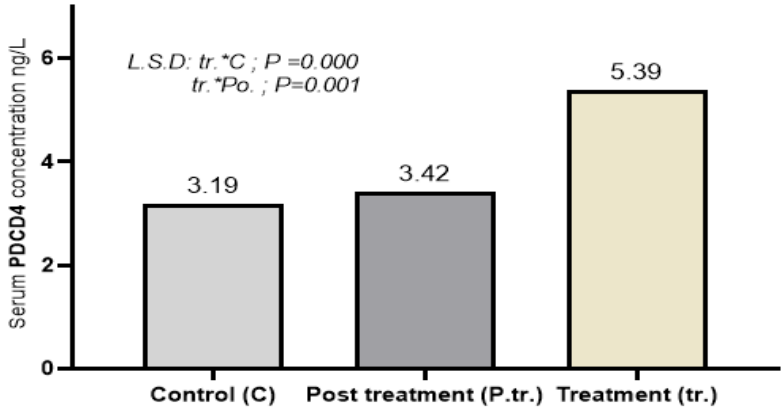

Figure 2: Serum *PDCD4* levels in breast cancer patients with different treatment status (during and post-treament) in comparison to healthy controls

The impact of age and BMI on PDCD4 protein concentration in serum.
Figure 3 demonstrated that there is no significant effect of both age and BMI on the PDCD4 protein concentration in serum in the present study.

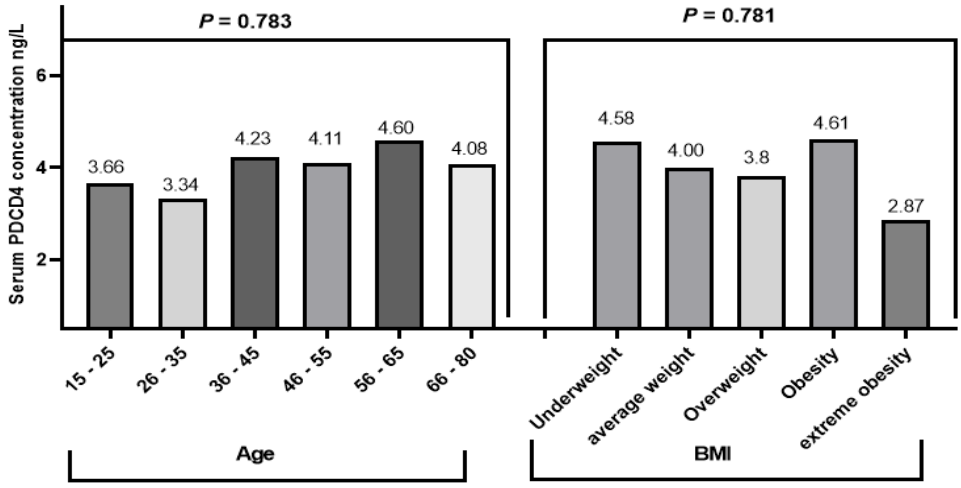

Figure 3: Age and BMI groups with PDCD4 protein concentration in serum in study subjects

The effect of breastfeeding, contraceptive, and family history on PDCD4 protein concentration in serum.
Results showed a significant (P=0.026) effect of breastfeeding on the PDCD4 protein concentration in serum, where the protein concentrations of the breastfeeding group and the non-breastfeeding group were 4.40 ng/L and 3.25 ng/L respectively. There is no significant effect of contraceptive and family history of cancer on the concentration of PDCD4 protein in the investigated subjects (Figure 4).

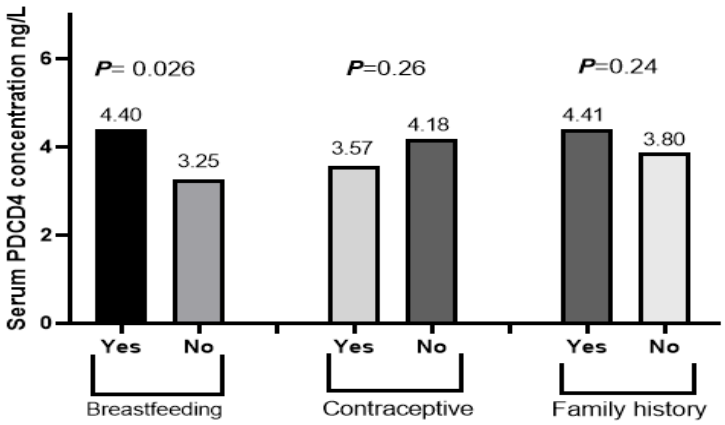

Figure 4: Breastfeeding, contraceptive, and family history with PDCD4 protein concentration in serum

Discriminatory power of PDCD4 protein in breast cancer

The results suggest that PDCD4 protein has very limited ability to distinguish between breast cancer patients and healthy control women. When assessing the discriminatory accuracy of PDCD4 levels, the area under the receiver operating characteristic curve (AUC) value was calculated to be 0.039. On the other hand, it shows some reasonable discriminatory ability when distinguishing between the treatment group of breast cancer patients and healthy control women (AUC of 0.7), Figure 5.

A **B**

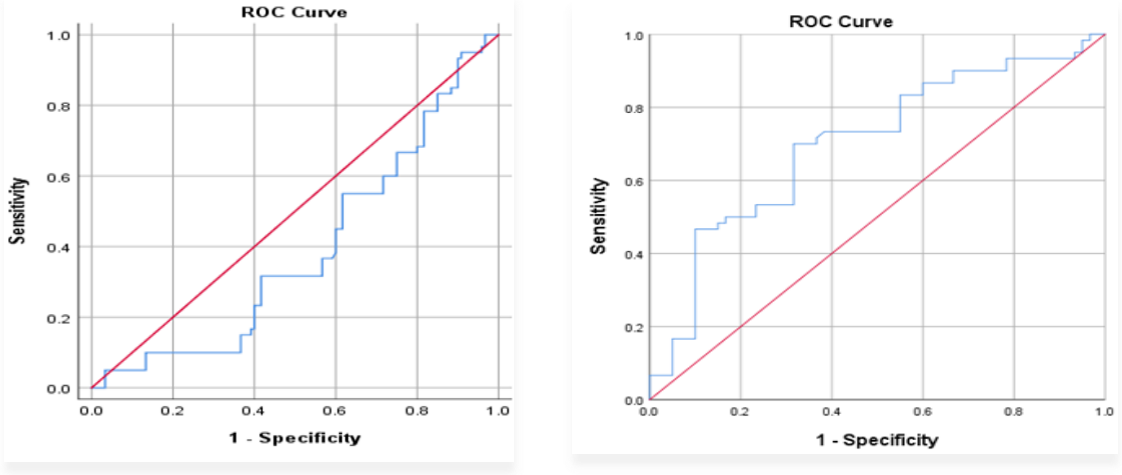

Figure 5: ROC curve for PDCD4 protein between, A: breast cancer patients and healthy control women,B: between pretreatment group of breast cancer patients and healthy control group women.

Table 2: Area under the ROC Curve for PDCD4 protein concentration between, A: breast cancer patients and healthy control women. B: between pre-treatment group of breast cancer patients and healthy control group women.

	Test Result Variable	AUC	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
					Lower Bound	Upper Bound
A	PDCD4	0.389	0.043	0.011	0.304	0.474
B	PDCD4	0.7	0.048	0.000	0.605	0.795

There could be a number of potential reasons for these results. It is possible that the levels of PDCD4 protein are not significantly different between breast cancer patients and healthy individuals, leading to the relatively low AUC in scenario A. However, in scenario B, the treatment group may have some effects on PDCD4 levels, resulting in a better discriminatory performance (AUC= 0.7) Table 2.

Discussion

Multiple single-nucleotide polymorphisms (SNPs) located in the intergenic regions are thought to be associated with breast cancer risk and linked to different aspects of breast carcinogenesis. Among Iraqi population the breast cancer where at the top of the list of malignancies-affecting women

The results of this study showed that individuals carrying the heterozygous AG genotype of the miR-449b rs10061133:A>G single nucleotide polymorphism had significantly higher levels of PDCD4 protein compared to those with the homozygous AA or GG genotypes. It seems possible that this could be due to the fitness-related heterosis resulting from evolutionary processes. Heterozygote advantage, also known as overdominance, comes into play when the heterozygous genotype of miR-449b rs10061133:A>G exhibits greater fitness concerning PDCD4 concentration than either of the homozygous genotypes. Heterosis refers to the phenomenon where a hybrid population exhibits higher fitness than an inbred population, and this has been previously explained through Mendelian dominance or overdominance, assuming a simple genotype-phenotype relationship [23]. In this study, the focus was investigated heterosis in the context of fitness and phenotypic variance in a system involving interacting genes, following by analyzing the protective value of PDCD4 protein concentration in individuals compered to heterozygous AG genotype.

An escalating quantity of studies has discussed the potential utilization of miRNAs as indicators for various medical conditions. To illustrate, a recent study has demonstrated that serum miRNAs maintain remarkably high diagnostic sensitivity and specificity, both exceeding 90%, for a range of cancer types, including breast, bladder, lung, ovarian, and prostate cancer[24].

Several studies support this assertion, indicating that a variety of regulators and biological processes, such as non-coding RNAs, proteasomes, estrogen, natural compounds, and inflammation [25], play roles in regulating PDCD4 expression in breast cancer. Previous research has also associated reduced PDCD4 expression with the acquisition of drug resistance in breast cancer. Studies examining aromatase inhibitor-resistant breast cancer cells found that activation of the HER2 oncogene resulted in downregulated PDCD4 expression levels. The proposed mechanism involved HER2 stimulation of the MAPK, AKT, and miR-21 signaling pathways, which in turn led to decreased PDCD4 expression. Furthermore, manipulating the microRNA/PDCD4 axis could be an effective approach for overcoming chemotherapy resistance in breast cancer. Notably, reduced PDCD4 expression is significantly correlated with shorter overall survival in breast cancer patients, suggesting that PDCD4 might serve as an independent prognostic marker for this disease [26].Through these data, it can be concluded that the treatment increases the process of programmed death of cancer cells, and this can be seen through the high protein concentration during the treatment period and its return to its similar concentrations in the normal state after the end of the treatment.

To explain the increased PDCD4 protein concentration in the serum of the studied breast cancer patients, especially in contrast to chemotherapy than in healthy control group, one

must first know the role of PDCD4 protein in general and immunologically in particular[27]. Furthermore, the PDCD4 protein has the ability to hinder the JNK and MEK/ERK pathways by directly binding to the MAP4K1 promoter. This inhibition leads to a reduction in the proliferation, migration, and invasion of cancer cells, while also promoting apoptosis [28]. Additionally, *PDCD4*, as a gene involved in apoptosis, can interact with eukaryotic initiation factor-4A (eIF4A) through its MA3 protein domain. This interaction results in the suppression of the combination of eIF4C with eIF4A, ultimately leading to a reduction in ribosome synthesis and a disruption in protein production [29].

To a certain extent, these findings align with a previous study conducted on early-stage oral squamous cell carcinoma. The research reported that in the tumor center, there was no correlation between the expression of both PDCD4 and eIF4A1 and factors such as age, smoking habits, and T-stage [30]. Most studies take into consideration the impact of body mass index (BMI) and its association with various diseases' aspects, given that this factor has a significant impact on the physiology of the human body [24]. The present study findings suggest that obesity does not seem to have a significant effect on the serum level of PDCD4 in comparison to those with healthy BMIs. This may be due to the interference of the effect of cancer or treatment on the outcome.

The present study found a significant association between breastfeeding and higher concentrations of the PDCD4 protein in women. As PDCD4 is thought to act as a protective factor against certain cancers, this suggests breastfeeding may help elevate protective mechanisms. This finding aligns with previous research demonstrating breastfeeding can lower risk of related cancers in both mother and child. This suggests that there are positive implications for encouraging women to opt for breastfeeding. The negative correlation between breastfeeding and the risk of specific maternal cancers can be explained by various biological mechanisms. One such mechanism is that breastfeeding can reduce exposure to endogenous estrogens, ultimately lowering the risk of maternal cancers. Another potential mechanism is that breastfeeding might aid in the removal of cells with damaged DNA through the excretion of human milk, thus reducing susceptibility to mutations. Additionally, breastfeeding has the ability to decrease insulin levels in women's serum, subsequently reducing serum concentrations of insulin-like growth factor IGF-1, which could impact the proliferation and anti-apoptosis of malignant cells[31].

In this study, breastfeeding showed to be linked to the increased of PDCD4 protein levels. This finding supports the notion that breastfeeding retains protective value against breast cancer. One potential explanation of such effects is the presence of some key substances produced in milk that have the ability to activate programmed death and regeneration of mammary cells. Alpha-lactalbumin is a crucial element in breast milk. Research has demonstrated that HAMLET (human alpha-lactalbumin made lethal to tumor cells) can trigger cell death in both tumor and bacterial cells. HAMLET induces apoptosis specifically in tumor cells, and normal differentiated cells are not susceptible to its effects[32].

The Area Under Curve (AUC) is a common metric utilized for assessing the performance of binary classifiers. In this context, it was employed to compare how well the PDCD4 protein discriminates between two different states: firstly, between breast cancer patients and healthy control women, and secondly, between the treatment group of breast cancer patients and healthy control women. The AUC value falls within a range of 0 to 1, where 0.5 signifies a classifier that performs no better than random chance, indicating an inability to distinguish between the two groups.

In the first state, the AUC was notably below 0.5, implying that the PDCD4 protein does not efficiently differentiate between breast cancer patients and healthy individuals. In the second state, an AUC of 0.7 proposes a reasonable ability of the PDCD4 protein to differentiate between the treatment group of breast cancer patients and healthy control women. Though 0.7 exceeds random chance (0.5), it still shows that the PDCD4 protein's performance in this condition is not enough for highly accurate discrimination. However, it can serve as a valuable option for assessing the response to cancer treatment, as it remains an important indicator of cancer recovery.

These findings should be advanced with attention, taking into account other factors that could affect the AUC, together with sample size and data accuracy. Moreover, it would be advantageous to carry out additional research and confirm these results using more extensive and varied datasets to arrive at more conclusive insights regarding the PDCD4 protein's ability to discriminate between these scenarios.

Conclusions

The findings from our study indicate that genetic variations in PDCD4 (rs6585018:G>A), miR-21 (rs1292037:T>G), and miR-449b (rs10061133:A>G) single nucleotide polymorphisms may play an important role in a woman's susceptibility to breast cancer. Specifically, our results suggest these genotypes are linked to individual differences in resistance against the disease. However, it is important to note that breast cancer has a multifactorial etiology, and genetic factors alone should not be depended upon to determine risk. Indeed, breastfeeding showed to increase the level of programmed cell death protein 4 (PDCD4) and both could be adapted in breast cancer prevention and management strategies.

Ethical approval

The research conducted was approved by the Ethical Approval Committee (Reference No. 22 dated 1/30/2023). The University of Anbar, College of Science approved this study.

Conflict of interest statement

The authors declare they have no conflict of interest.

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