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The role of hypoxia-inducible factor 1 alpha and peroxisome proliferator activated receptor gamma coactivator 1 alpha in the diagnosis of breast cancer

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ABSTRACT

This study aimed to assess the possible association between hypoxia-inducible factor 1 (HIF-α1) and proliferator activated receptor gamma coactivator 1 (PGC-α1) levels in early and advanced breast cancer patients and to study the correlation between these parameters in a case-control study conducted on 40 females with breast cancer categorized into early and advanced stages, with 20 patients in each group, collected from the Medical City and Oncology teaching hospital, Baghdad, Iraq, between June and October 2023. The levels of HIF-α1 and PGC-α1 were measured in the serum of breast cancer patients by ELISA technique and compared with 40 age- and gender- matched controls, which showed that the levels of HIF-1α and PGC-1α increased significantly in the early and advanced stages in comparison with controls, which indicates that the markers can be used as diagnostic markers for breast cancer in the early and advanced stages.

Keywords: HIF-1α, PGC-1α, breast cancer, diagnostic markers

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INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and a major cause of death in women worldwide, diagnostic marker for breast cancer typically involves a combination of various tests and procedures[1]. However, there are several key markers and tests commonly used in the diagnostic process for breast cancer such as carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), carbohydrate antigen 125 (CA125), carbohydrate antigen 199 (CA199) and carbohydrate antigen 153 (CA153)[2]. Mammography is a standard screening tool for detecting breast abnormalities and breast ultrasound is frequently employed alongside mammography to differentiate between solid masses and fluid-filled cysts which enhances the diagnostic capabilities. Breast Magnetic Resonance Imaging (MRI) can provide detailed images of the breast and is often used for further evaluation, especially in high-risk cases in addition to Biopsy which is considered as definitive diagnosis of breast cancer[3]. Both Fine Needle Aspiration Cytology (FNAC) and Tru-Cut biopsy demonstrate commendable diagnostic accuracy. However, Tru-Cut biopsy exhibits higher accuracy compared to FNAC for detecting this pathological condition[4].

Circulating Tumor Markers are generally not specific enough for a definitive diagnosis, for example CEA is not specific to breast cancer, and elevated levels can also be seen in other conditions, such as colorectal cancer. CEA is more commonly used in monitoring the progression of the disease and evaluating the response to treatment rather than for the initial diagnosis[5]. Another example is CA 15-3 which is a protein that may be elevated in the circulation of breast cancer patients, particularly those with advanced or metastatic disease. CA 15-3 also not specific to breast cancer and can be elevated in other conditions include endometriosis, pelvic inflammatory disease and liver disease. It can also be increased during pregnancy[6]. CA 15-3 is often used as a tumor marker for monitoring disease progression and treatment response rather than for diagnostic purposes[7].

When cells encounter low levels of oxygen (Hypoxia)[8], HIF plays a pivotal role in coordinating cellular reactions to hypoxia by controlling the expression of genes associated with oxygen homeostasis, angiogenesis, and glycolysis which is crucial for cells to cope with this challenges[1]. Even under normoxic (normal oxygen) conditions, the heightened expression of HIF-1α in breast tumors is evident, indicating that the irregularities in HIF-1α regulation within these tumors are not exclusively tied to low oxygen levels[9]. The peroxisome proliferator-activated receptor gamma coactivator 1-alph (PGC-1α) functions as a co-activator for steroid and receptor of nuclear, participating in adaptive ther-

mogenesis, fatty-acid oxidation, energy metabolism, thyroid hormone receptors, homeostasis of cellular cholesterol, and gluconeogenesis[10]. The processes underlying tumor invasion, proliferation, progression, and metastatic potential remain not clear, but recent findings suggest that in invasive tumors, tumor cells primarily rely on mitochondrial respiration. In this context, oxidative phosphorylation activated processes by PGC-1α appears to play a significant role[11]. The correlation between PGC-1α and HIF-1α has undergone thorough examination, revealing that in the context of angiogenesis, PGC-1α induces vascular endothelial growth factor (VEGF) expression without reliance on HIF-1α. Nevertheless, it can be postulated that PGC-1α-induced mitochondrial respiration may lead to reduced oxygen levels and an augmentation in the production of reactive oxygen species (ROS)[12].

This study aimed to assess the possible association between Hypoxia-Inducible Factor1 HIF-α1 and proliferator activated receptor gamma coactivator PGC-α1 level with early and advance breast cancer patients, and to study the correlation between these parameters.

METHODOLOGY

Study design

This study is case-control research conducted at Al-Nahrain University's College of Medicine, department of chemistry and biochemistry. The research proposal was authorized by the Al-Nahrain University College of Medicine's Ethical Committee. Forty female Iraqi patients with breast cancer were documented by histopathology and collected in from Al- Oncology teaching hospital Medical City, Baghdad, Iraq. Blood samples were obtained from all patients upon their consent, following hospitalization and before the initiation of any medication. The study was conducted between May 2022 and December 2022. All participants included in the study were aged between 18 and 60 years.

1. Control group: Includes 40 samples of apparently healthy-aged and sex-matched volunteers.

2. Case group: Includes 40 (18 early, 22 advance stage) samples with confirmed breast cancer, diagnosed by true cut histopathology.

Exclusion criteria

Female with tumor other than breast cancer, pregnant and lactating women, viral infected women, women who exposure to radiotherapy and chemotherapy taken.

Blood sample collection and storage

Approximately 5 ml of blood samples were collected from the participants. The blood was allowed to clot at room temperature for 15 minutes, and then the serum was separated by centrifugation at 3000 rpm for 10 minutes. The isolated serum was stored in a -4°C freezer until it was ready to be used in the study, Human HIF-1a and PCG-1a kit was measured using Elisa Human Reader, purchased from Cloud-Clone Corp Company, USA. Other tests include urea, creatinine, ALT, AST, ALP.

Statistical analysis

The study data were analyzed utilizing SPSS software version 20. Numeric variables were presented as mean, standard error (SE) and standard deviation (SD), and statistical comparisons were conducted through ANOVA, followed by the post-hoc Tukey test. A significance level of $p \leq 0.05$ was considered statistically significant. Categorical variables were expressed as numbers and analyzed using cross-tabulation to evaluate the frequency and percentage of each variable within the studied groups.

RESULTS and DISCUSSIONS

Age and body mass index (BMI) of the studied groups were summarized in Table 1 which showed non-significant differences in age and BMI among all studied groups.

Table 1. Age and BMI of the patients with early and advance stages in comparison with controls

		N	Mean	SD	SE	p^a	p^b	p^c	p^d	p^e
Age	Control	40	43.4	6.62	1.48	0.923	0.972	0.992	0.926	0.708
	Early Stage	22	42.62	5.25	1.46					
	Advance Stage	18	42.91	4.46	1.34					
	All Patients	40	42.75	4.8	0.98					
BMI	Control	40	26.88	1.24	0.28	0.397	0.137	0.75	0.131	0.058
	Early	22	27.63	2.06	0.57					
	Advance	18	28.13	1.49	0.49					
	All Patients	40	27.84	1.83	0.39					

P^a value between controls and early-stage patients; P^b value between controls and advance-stage patients; P^c value between early- and advance-stage patients; P^d value among controls, early- and advance-stage patients (ANOVA test); P^e value between controls and all patients; SD: Standard deviation; SE: Standard error.

Table 2 showed that the levels of HIF-1α and PGC-1α increased significantly ($p<0.001$) in the early and advanced stages in comparison with controls, and these two subgroups were non-significantly different from each other. On the other hand, CEA and CA 15-3 showed a different manner of increment in that the levels of these markers increase non-significantly in early stages in comparison with controls whereas advanced stages patients showed the significant increase in comparison with both controls and early stages patients with breast cancer.

Table 2. Levels of HIF-1α, PGC-1α, CEA and CA 15-3 in all studied groups

		N	Mean	SD	SE	Pa	Pb	Pc	Pd	Pe
HIF-1α	Control	40	1.58	0.31	0.07	<0.001	<0.001	0.821	<0.001	<0.001
	Early	22	3.91	1.59	0.44					
	Advance	18	4.22	1.87	0.56					
	All Patients	40	4.05	1.69	0.35					
PGC-1α	Control	40	1.07	0.1	0.02	<0.001	<0.001	0.944	<0.001	<0.001
	Early	22	2.62	1.01	0.28					
	Advance	18	2.72	0.97	0.29					
	All Patients	40	2.67	0.97	0.2					
CEA	Control	40	0.51	0.2	0.04	0.275	<0.001	0.004	<0.001	0.001
	Early	22	1.2	0.48	0.13					
	Advance	18	2.94	2.43	0.73					
	All Patients	40	1.99	1.86	0.38					
CA 15-3	Control	40	13.27	4.77	1.07	0.938	0.002	0.011	0.002	0.482
	Early	22	14.05	3.94	1.09					
	Advance	18	22.14	10.47	3.16					
	All Patients	40	17.76	8.53	1.74					

P^a value between controls and early-stage patients; P^b value between controls and advance-stage patients; P^c value between early- and advance-stage patients; P^d value among controls, early- and advance-stage patients (ANOVA test) P^e value between controls and all patients; SD: Standard deviation; SE: Standard error.

Results illustrated in Table 3 showed that PGC-1α levels were positively and significantly correlated with ALP (r=0.470, p=0.02) and with CEA (r=0.467, p=0.021). Additionally, CEA and CA15-3 were also correlated positively and significantly (p=0.624, p=0.001).

Table 3. Correlation among all studied groups in patients with breast cancer

		BMI	Urea	Cr	ALT	AST	ALP	HIF-1α	PGC-1α	CEA	CA 15-3
HIF-1α	r	-0.002	0.285	-0.011	-0.24	0.056	-0.051	-	-0.234	0.106	0.309
	p	0.994	0.177	0.96	0.258	0.795	0.811	-	0.27	0.621	0.142
PGC-1α	r	-0.054	0.346	0.08	0.311	0.198	0.470*	-0.234	-	0.467*	0.304
	p	0.813	0.098	0.709	0.139	0.354	0.02	0.27	-	0.021	0.148
CEA	r	0.112	0.367	0.144	0.02	0.361	0.084	0.106	0.467*	-	0.624**
	p	0.619	0.078	0.503	0.926	0.083	0.695	0.621	0.021	-	0.001
CA 15-3	r	0.308	0.192	0.012	0.17	0.134	0.049	0.309	0.304	0.624**	-
	p	0.164	0.368	0.955	0.427	0.532	0.819	0.142	0.148	0.001	-

r: Pearson correlation coefficient; p: Significance; Cr: Creatinine.

Table 4 and Figure 1 showed that PGC-1α showed the highest AUC of 1 with sensitivity and specificity of 100% in patients with breast cancer when compared with controls, followed by CEA that showed AUC, sensitivity, and specificity of 0.972, 91.7%, and 85%, respectively, and HIF-1α with 0.958, 91.7%, and 100%, respectively. The only marker that showed a low value was CA 15-3 with 0.704, 66.7%, and 70%, respectively. An interesting finding is that CEA when combined with CA 15-3 provides a higher AUC of 0.981 with perfect sensitivity (100 %) and excellent specificity (91.7).

Table 4. ROC curve results of HIF-1α, PGC-1α, CEA and CA 15-3 between breast cancer patients' group and controls

Parameters	AUC	Cut-Off value	Sensitivity (%)	Specificity (%)
HIF-1α	0.958	2.433	91.7	100
PGC-1α	1.000	1.24	100	100
CEA	0.972	0.745	91.7	85
CA 15-3	0.704	14.35	66.7	70
Combination of CEA and CA 15-3	0.981	-	100	91.7

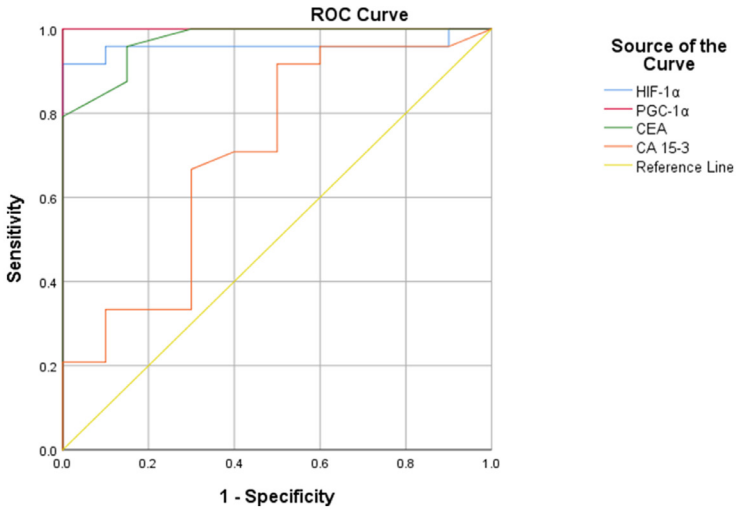

Figure 1. ROC curve results of HIF-1α, PGC-1α, CEA and CA 15-3 between breast cancer patients and controls

These markers are not definitive for breast cancer diagnosis, and decisions regarding treatment and monitoring should be made based on a comprehensive assessment by healthcare professionals so that not used as diagnostic markers. In Hypoxic Microenvironment Rapid tumor growth in the early stages can outpace the development of new blood vessels, leading to areas of inadequate oxygen supply or hypoxia[13]. HIF-1α is highly responsive to low oxygen levels, and its stabilization occurs in response to hypoxia. In early breast cancer, regions of hypoxia trigger the accumulation and activation of HIF-1α. The early tumor microenvironment relies on angiogenesis to provide the necessary blood supply for sustained growth HIF-1α triggers the production of pro-angiogenic molecules, including Vascular Endothelial Growth Factor (VEGF). Promoting the formation of new blood vessels to support the growing tumor[14].

In advanced breast cancer, the tumor outgrows its existing blood supply, resulting in persistent and widespread hypoxia. The chronic hypoxic conditions contribute to sustained activation and accumulation of HIF-1α in cancer cells. Metastatic Spread in advanced breast cancer is often characterized by increased metastatic potential. HIF-1α is involved in the induction of genes associated with invasion and metastasis, facilitating the spread of cancer cells to distant organs. The microenvironment of advanced tumors undergoes dynamic changes, including fluctuations in oxygen levels[15]. These changes can further activate HIF-1α as cancer cells adapt to the evolving conditions. Therapeutic Resistance to treatment modalities, such as chemotherapy and radiation, can

induce hypoxia in tumor regions. In response to these therapies, cancer cells may upregulate HIF-1α as a survival mechanism, contributing to resistance against treatment[16]. PGC-1α is known for its role in regulating cellular energy metabolism. In cancer cells, including breast cancer, there can be metabolic adaptations to support the increased energy demands of rapidly dividing cells. Elevated PGC-1α might contribute to these adaptations[17]. PGC-1α is a key regulator of mitochondrial biogenesis and function. Breast cancer cells often exhibit changes in rely on mitochondrial metabolism to fulfill their energy requirements. Increased PGC-1α expression may be a part of these adaptations[18].

PGC-1α is involved in the cellular response to oxidative stress. Breast cancer cells may experience higher levels of oxidative stress due to various factors, and an elevation in PGC-1α could be part of the cellular response to mitigate oxidative damage.PGC-1α expression can be influenced by hormonal signaling pathways. Breast cancer, especially hormone receptor-positive subtypes, is influenced by hormonal factors. Changes in hormonal signaling in breast cancer cells may contribute to alterations in PGC-1α expression[19]. Dysregulation of genes involved in the PGC-1α pathway through genetic mutations or epigenetic modifications might contribute to elevated PGC-1α expression in breast cancer[20]. In the early stages of breast cancer, alterations in cellular metabolism and mitochondrial function may be critical for tumor initiation and growth[21,22]. Elevated PGC-1α might support these early metabolic adaptations. In advanced stages, where tumors become more aggressive and may develop resistance to treatment, PGC-1α could contribute to sustaining the high energy demands of rapidly dividing cancer cells and promoting cell survival [23,24].

This study shows that the increasing in Hypoxia-Inducible Factor1 Alpha and Peroxisome Proliferator Activated Receptor Gamma Coactivator 1 Alpha (HIF-1α and PGC-1α) associated with breast cancer in early and advance stage, positive significant correlations were demonstrated between age and PGC-1α, HIF-1α and AST. That PGC-1α showed the highest AUC with sensitivity and specificity of 100% in patients with breast cancer when compared with controls, followed by HIF-1α.

STATEMENTS OF ETHICS

The study received approval from the "Institute Review Board (IRB) of Al-Nahrain University/College of Medicine" on April 30, 2023 (38/2023) with a number 20221276.

CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTIONS

Design – Abdul-Rasheed OF, Alsammarraie AZ; Acquisition of Data – Algburi DYR, Abdulhassan BA, Alsammarraie AZ; Analysis of Data – Algburi DYR, Abdulhassan BA; Drafting of the Manuscript – Algburi DYR, Abdulhassan BA, Abdul-Rasheed OF; Critical Revision of the Manuscript – Abdulhassan BA, Abdul-Rasheed OF, Alsammarraie AZ; Statistical Analysis – Algburi DYR, Alsammarraie AZ; Technical or Financial Support – Algburi DYR, Abdulhassan BA, Abdul-Rasheed OF; Supervision – Abdulhassan BA, Abdul-Rasheed OF, Alsammarraie AZ.

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