RESEARCH ARTICLE

Editorial Process: Submission:01/23/2025 Acceptance:09/07/2025 Published:09/12/2025

Impact of Chronic Stress and Anxiety on Interleukin-6, Hypoxia-Inducible Factor-1α and on Pathological and Immunohistochemical Parameters in Breast Cancer

Yahia Saddek[1]*, Zineb Tahari[1,2], Asmahane Medjdoub[1,2], Nadia Bessaih[3], Moussa Messatfa[4,5], Miloud Lahmer[6], Sonia Seddiki[1], Tewfik Sahraoui[1]

Abstract

Objective: The Aim of this study is to evaluate stress and anxiety by rating scale in breast cancer (BC) patients and their association with interleukin-6 (IL-6), hypoxia-inducible factor-1α (*HIF-1α*), pathological and immunohistochemical parameters. **Methods:** We analyzed serum concentrations of IL-6 and *HIF-1α* in 69 BC patients from western Algeria using ELISA technique, with optical density measured at a wavelength of 450 nm. Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (*HER2*), and *Ki-67* expression were assessed by immunohistochemistry. Venous blood samples were collected in heparinized tubes. Additionally, stress and anxiety levels were evaluated using standardized questionnaires and compared with the other measured parameters. *Results:* Analysis of the correlations between the various variables revealed a positive correlation between anxiety and *HIF-1α* ($p = 0.049$, pearson correlation coefficient [PC] = 0.24), as well as between anxiety and the Scarff-Bloom-Richardson (SBR) grade ($p = 0.037$, PC = 0.26). A negative association was also observed between *HIF-1α* and *HER2* status ($p = 0.02$, with the mean *HIF-1α* level in *HER2*-positive patients being 0.01 compared to 0.27 in *HER2*-negative patients). No significant association was found between IL-6 and stress ($p = 0.4$), nor between IL-6 and anxiety ($p = 0.28$). **Conclusion:** Our results show a positive correlation between anxiety and *HIF-1α*, which plays a crucial role in tumor angiogenesis, as well as with SBR grade. These findings suggest that anxiety may modulate tumor angiogenesis and contribute to increased tumor aggressiveness and grade. A negative association was also observed between *HER2* and *HIF-1α*, which may be explained by a negative regulatory mechanism between the *HER2* and *HIF-1α* signaling pathways. Furthermore, no association was found between stress or anxiety and IL-6 levels.

Keywords: Anxiety- breast cancer- hypoxia-inducible factor-1α- interleukin-6- stress

Asian Pac J Cancer Prev, **26 (9)**, 3271-3279

Introduction

Breast cancer (BC) is a heterogeneous disease with different morphological appearances, molecular characteristics, behavior and response to treatment [1], with an estimated 2.3 million new cases occurring and approximately 685 000 deaths, it was responsible for 16% of cancer deaths in female individuals in 2020 [2].

Only about 50% of breast carcinomas can be attributed to a physiological cause or genetic risk factor [3]. In addition, as a multifactorial disease, the etiologies of BC include not only distinct intrinsic factors such as genetic status, but also environmental factors such as obesity, lifestyle, chronic inflammation and psychological stress [4].

The role of stress in the etiology of BC is the subject of considerable interest. In addition, a potential relationship between stress and BC risk has been examined in studies of differing design with contradictory results [5]. The stress caused by such events can affect the hypothalamic-pituitary-adrenal axis (HPA) and cause disturbances in the endocrine system, increasing cortisol levels and inhibiting antineoplastic activity [6]. Furthermore, high anxiety, followed by stressful events, increases the risk of BC through intermediate parameters such as inflammatory markers, cytokines, and interleukins by suppressing the

*[1]Biology of Development and Differentiation Laboratory, Ahmed Ben Bella Oran, University, Oran, Algeria. [2]Department of the Living and Environment, University of Sciences and Technology of Oran, Mohamed-Boudiaf, Oran, Algeria. [3]Department of Medical Oncology, University Hospital Establishment of Oran, Faculty of Medicine, Ahmed Ben Bella Oran 1 University, Oran, Algeria. [4]Faculty of Medicine, Abdelhami Ibn Badis University Mostaganem, Mostaganem, Algeria. [5]Immunology Laboratory, Pasteur Institute of Oran, Oran, Algeria. [6]The National Center of Research in Social and Cultural Anthropology, Oran, Algeria. *For Correspondence: saddek.yahia@gmail.com*

immune system [7]. Persistent activation of the HPA axis in the response to chronic stress and in depression probably alters the immune response and contributes to the development and progression of certain types of cancer [8]. Other research showing that acute and chronic psychological events also increase interleukin-6 (*IL-6*) concentrations confirms the existence of a close link between an organism's response to physiological and psychological disturbance [9] , while other studies also show that stress and anxiety can modulate tumor angiogenesis. However, due to the multiplicity of processes in a complex microenvironment, the role of psychological events in tumor angiogenesis and how they contribute to tumor progression are not fully elucidated [10].

The accumulated understanding of stress and its mechanism of action during the 20th century, combined with enthusiasm about the impact of stress hormones on the development of cancer, has given rise to a great deal of research. Some strong but tentative hypotheses have emerged in support of psychological events and stress-induced carcinogenesis. However, the results of epidemiological studies are contradictory, ranging from no association to an association between stress and BC [11].

The main objective of this study is to evaluate stress and anxiety by a rating scale in BC patients from western Algeria and their association with the immune system by examining serum *IL-6* concentration and also the relationship with tumor angiogenesis by analysis of hypoxia-Inducible Factor-1α (*HIF-1α*). We also investigated their association with pathological, immunohistochemical (IHC) parameters such as human epidermal growth factor receptor 2 (*HER2*), estrogen receptor (ER), progesterone receptor (PR) and cell proliferation marker K-i67, as well as lymph node number (LN).

Materials and Methods

Population

The study was conducted on 69 patients newly diagnosed with BC between 2020 and 2022. Our patients, from western Algeria, were informed of the conditions of the study and gave their informed consent to have their blood drawn for our tests, as well as for the administration of our stress and anxiety questionnaires.

Methods

Venous blood samples were collected in a heparinized tube and centrifuged at 3000 rpm for 15 min. The plasma obtained was conserved at –80°C until measurements were made. The dosage of *IL-6* and *HIF-1α* established in biology of development and differentiation laboratory (Ahmed Ben Bella Oran 1 University) and Pasteur Institute of Algeria, Oran Branch (immunology laboratory). IHC parameters such as *ER, PR*, *HER2* and *Ki-67* were performed in the anatomopathology laboratory of EHU Oran. Other pathological parameters such as LN count and tumor size were collected from medical records. Socio-demographic information, anthropometric characteristics and personal history were collected using a baseline patient questionnaire. Stress and anxiety questionnaires were administered to patients to assess their levels and compare them with other parameters, it is important to note that the questionnaires on Stress, and Anxiety were administered to patients during their disease, whereas the survey questions focused on these psychological factors before the onset of the disease.

Self-rated stress scales

The concept of stress is important in clinical practice for diagnosis and treatment: a scale that has been used satisfactorily in clinical practice for several years by general practitioners, occupational physicians and psychiatrists [12]. The stress questionnaire includes 11 items exploring 11 groups of possible reactions to stress. The subject evaluates the importance of the reaction with a score ranging from 1 (very little reaction) to 6 (extremely important reaction). The score range is 11 to 66.

Anxiety assessment questionnaire

This anxiety questionnaire comprises 8 items, the importance of each of which is assessed on a scale of 0 to 3. If the total exceeds 10 points, the person probably suffers from marked anxiety [13].

Protocol Elisa sandwich test for IL-6 assay (HUMAN IL-6 ELISA KIT SIGMA - RAB0306) and HIF-1α (HUMAN HIF-1α ELISA KIT SIGMA - RAB 1057)

According to manufacturer's recommendations the suggested dilution for the serum of the normal plasma sample is 2-fold. A volume of 100 ul of each standard and sample are added to the appropriate wells. Incubation is performed for 2.5 hours at room temperature. The wells are washed four times with 300 ul of wash solution followed by adding 100 ul of detection antibody in each well. A second incubation is performed for 1 hour at room temperature with gentle shaking. In the same way as before, the wells are washed, and then 100 µl of streptavidin solution is added to each well and incubated for 45 minutes at room temperature with gentle shaking. After a final wash, a solution of 100 µl of TMB substrate reagent is added to each well. The final incubation is performed for 30 minutes at room temperature in the dark with gentle shaking. The reaction is stopped by adding 50 µl of stop solution. The optical density is measured at a wavelength of 450 nm. (same steps and protocol for *HIF-1α* and *IL-6* assays).

Immunohistochemical study

IHC, which originated in 1942 with the publication of Coons et al. [14], is a method for detecting proteins or other antigens in tissue sections. The aim of this technique in our work is to determine the quantity of *HER2*, PR, ER and *Ki-67* proteins present in BC cells.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 30.0 was used for the statistical analysis. A descriptive analysis of pathological and IHC parameters was performed. Pearson correlation was used to compare quantitative parameters including *IL-6*, *HIF-*

1α, stress and anxiety scale, *Ki-67*, SBR grade and LN count. Independent samples T-test was used to look for association between qualitative parameters including *HER2*, ER, PR, and quantitative parameters previously reported. $P < 0.05$ was considered statistically significant.

Results

The results of the descriptive analysis show that 77% of cases are *HER2* positive versus 23% negative, more than half of ER and PR hormone receptors are positive with 66% and 59% respectively. The right breast represents 49.3%, the left 47.8% and bilateral represents 2.9%. 7.2% of our patients are single versus 92.8 married. 81% of women were breastfeeding versus 19% who did not breastfeed. Patients aged 50 or over represented 47.8%, and those over 50 represented 52.2%. BC with a family antecedents (FA) were identified in 52.4% of cases, while 48.6% of BC were identified without a FH. more than half of our patients with tumor size T2 followed by T1, T3 and T4 respectively. The results of the descriptive analysis are shown in Table 1.

Association analysis of psychological (stress and anxiety), pathological parameters and other tumor markers with qualitative IHC parameters (*HER2*, ER, PR) showed a negative significant association between Hif-1a and *HER2*+ with $p = 0.02$, given that the mean concentration of Hif-1a in *HER2*+ is 0.01 and *HER2*- is 0.27. An association negative was also found between the cell proliferation protein *Ki-67* and ER with $p = 0.043$, the mean percentage of *Ki-67* in ER+ is 21.24 and ER- is 33.63. No significant association was found between other parameters and IHC parameters. The results of the analysis are presented in Table 2.

Correlation analysis between the various variables shows that there is a positive correlation between anxiety and stress with $P = 0.001$ and correlation coefficient (CC) = 0.57. Positive correlations were also found between anxiety and Hif-1a and also with SBR grade with $p = 0.049$, 0.037 and CC = 0.24, 0.26 respectively. A positive correlation was also found between lymph node count and *Ki-67* protein with $p = 0.047$ and CC= 0.31. BMI was positively correlated with tumor size and also lymph node count with $P = 0.043$, 0.03 and CC= 0.24, 0.26 respectively. A positive correlation was also found between lymph node count and *Ki-67* protein and also with tumor size with $p = 0.047$, 0.042 and CC= 0.31, 0.25 respectively. no significant correlations between other parameters. The results of the correlations are presented in the Table 3.

Discussion

Breast cancer (BC) is a heterogeneous disease and the most common cancer among women with varied morphological appearances, molecular features, behavior, and response to therapy, their management relies on the availability of robust clinical and pathological prognostic and predictive factors to guide patient decision making and selection of treatment options [1].

Clinical decision-making in BC is based on testing for ER and PR by IHC and *HER2* by IHC and/or fluorescence in situ hybridization (FISH) [15]. *HER2* overexpression has been associated with a more aggressive phenotype and reduced survival. *HER2*-positive patients account for 20-25% of all BC cases [16, 17]. In addition, approximately 70% of BC patients are ER-positive and 60% are PR-positive [15, 18]. Our results show that 23% of our patients are *HER2*-positive, 66% are ER-positive and 59% are PR-positive. these results are in line with previous data in the literature.

The size of BC at diagnosis has always been considered a fundamental determinant of clinical outcome. However, the tendency of certain BC subtypes to behave aggressively, despite their small size (≤1 cm in diameter), calls into question the principle that cancer size should always be considered in therapeutic decisions [19]. Babu K et al. noted that T2 tumor size accounted for more than half the cases, or 52.7% [20]. Another study by Subramaniam et al. found that the T2 tumor ranked first, followed by T1 and T3 respectively [21] . Our results are in line with previous findings in the literature.

Table 1. Descriptive Analysis of Immunohistochemical Parameters, Pathology and Other Variables

Parameters	Number (Pourcentage)
HER2	
Positive	16 (23)
Negative	53 (77)
ER	
Positive	46 (67)
Negative	23 (33)
PR	
Positive	41 (59)
Negative	28 (41)
Breast affected	
Right	34 (49.3)
Left	33 (47.8)
bilateral	2 (2.9)
Marital status	
Single	5 (7.2)
Married	64 (92.8)
Breastfeeding	
Yes	56 (81)
No	13 (19)
Age	
≥50	33 (47.8)
<50	36 (52.2)
Family antecedents	
Yes	36 (52.4)
No	33 (48.6)
Tumor size	
T1	12 (18)
T2	38 (55)
T3	11 (16)
T4	8 (11)

Table 2. Association Analysis of Pathological, Psychological and Other Tumor Marker Parameters with Immunohistochemical Parameters

Parametres	*HER2*		*ER*		*PR*	
	Positive	Negative	Positive	Negative	Positive	Negative
Stress						
Average	27.37	28.97	28.58	28.54	28.24	29.03
Standard deviation	5.99	6.52	6.73	6.28	6.28	6.63
P value	0.39		0.98		0.63	
IL-6						
Average	110.61	129.12	76.7	217.36	77.61	193.51
Standard deviation	194.21	349.97	172.77	480.25	181.28	442.44
P value	0.84		0.2		0.22	
Anxiety						
Average	10.87	10.65	10.78	10.59	10.91	10.42
Standard deviation	4.24	3.71	3.83	6.28	3.93	3.68
P value	0.84		0.85		0.61	
HIF-1α						
Average	0.01	0.27	0.15	0.37	0.17	0.31
Standard deviation	0.001	0.84	0.63	0.92	0.67	0.85
P value	0.02		0.27		0.45	
Ki-67						
Average	28.44	25.45	21.24	33.63	24.46	29.36
Standard deviation	14.42	19.29	14.55	21.62	16.92	20.9
P value	0.66		0.043		0.41	
Tumor size						
Average	1	2.1	2.14	1.95	2.15	1.96
Standard deviation	0.29	0.84	0.81	0.74	0.85	0.67
P value	0.64		0.37		0.31	
SBR grade						
Average	2.25	2.31	2.23	2.4	2.21	2.42
Standard deviation	0.44	0.55	0.48	0.59	0.47	0.57
P value	0.68		0.25		0.12	
Lymph node count						
Average	1.93	1.56	1.76	1.45	1.92	1.26
Standard deviation	1.08	2.72	2.74	2.21	2.08	0.47
P value	0.61		0.65		0.32	
body mass index (BMI)						
Average	27.77	28.3	28.25	27.99	28.44	27.74
Standard deviation	6.27	5.66	6.28	4.74	6.25	5.05
P value	0.75		0.85		0.63	

The incidence of BC varies from country to country, but genetics and family antecedents (FA) influence its incidence [22]. According to the results of a case-control study, a history of BC is associated with a two-fold increase in the risk of developing BC [23]. The researchers stated that the clinical management of BC in women with or without a FA of cancer and with knowledge of the underlying mutations should be no different. Having a family history of BC may be a candidate for chemoprevention with tamoxifen or intensified BC screening with magnetic resonance imaging [24]. Our results show that patients with a FA were identified in 52.4% of cases, which is a very high proportion. These results are consistent with previous literature showing that FA is an important risk factor in BC.

In our series, 49.3% of tumors were located in the right breast and 47.8% in the left breast, bilateral tumors were found in 2.9% of cases. This rate is comparable to that of El Fouhi et al. [25] with 50.2% of cases on the left, 44.7% on the right and 1.3% bilateral.

Breastfeeding is the biologically normative way of feeding human infants and reduces the risk of BC in mothers [26]. According to a report by the World Cancer Research Fund and the American Institute of Cancer

Table 3. Correlation Analysis between Different Quantitative Variables

Parametres	Body mass index (BMI)	Tumor size	lymph node count	SBR Grade	*Ki-67*	*HIF-1α*	Anxiety	Stress	*IL-6*
IL-6									1
Pearson correlation	-0.05	-0.13	-0.15	-0.08	0.13	-0.06	-0.135	0.26	
p-value	0.67	0.29	0.2	0.5	0.39	0.6	0.28	0.40	
Stress								1	
Pearson correlation	-0.03	-0.14	-0.15	0.009	-0.001	-0.09	0.57		
p-value	0.81	0.24	0.2	0.94	0.99	0.44	0.001		
Anxiety							1		
Pearson correlation	-0.11	-0.03	-0.17	0.26	0.01	0.24			
p-value	0.37	0.8	0.15	0.037	0.94	0.049			
HIF-1α						1			
Pearson correlation	0.02	0.1	-0.08	0.15	-0.07				
p-value	0.86	0.37	0.46	0.19	0.62				
Ki-67					1				
Pearson correlation	0.19	0.08	0.31	0.08					
p-value	0.22	0.57	0.047	0.58					
SBR Grade				1					
Pearson correlation	-0.01	0.11	0.12						
p-value	0.89	0.35	0.3						
lymph node count			1						
Pearson correlation	0.24	0.25							
p-value	0.043	0.042							
Tumor size		1							
Pearson correlation	0.26								
p-value	0.03								
Body mass index (BMI)	1								
Pearson correlation									
p-value									

Research, breastfeeding is beneficial to women's health and breastfeeding has been shown to reduce the number of cases of BC [27] . Analysis of our results shows that 78.1% of women breastfeed, compared with 21.9% of women who do not breastfeed. Our findings show that our patients maintain breastfeeding and that there may be risk factors other than breastfeeding associated with BC.

New risk factors have been studied, including psychological stress an imminent risk factor with a documented negative impact on neuro-endocrine and immune system [11]. BC is associated with symptoms which are physical, psychological, and cognitive. Prolonged conditions of stress, such as those caused by stressors, predispose the body to diseases such as BC. It has been suggested that BC is correlated with prolonged exposure to stress [28].

Psychological stressors are social and physical environmental conditions that challenge an organism's coping skills and resources [29]. A response to stress, caused by a real or imagined threat (stressors), can be described as an organism's state of anxiety in response to a challenge to its homeostasis [30]. During this state, the body initiates an integrated reaction comprising physiological and behavioral responses. Internal threats, or systemic stressors, include physical changes in the body, such as hypoglycaemia or hypovolaemia (reduced blood volume), which occur [31]. In addition, several studies have shown that stress and anxiety are significantly associated [30]. Our results are in line with the literature show that there is a positive correlation between stress and anxiety.

Psychological evaluation of anxiety in women with suspected or diagnosed BC can provide psycho-oncological evidence to predict disease progression and tailor specific oncological therapies. In addition, psycho-oncological assessments have shown that anxiety is a major risk factor for the unfavorable evolution of oncological disease [32]. Anxiety to some degree, triggering the release of catecholamines by the body. The b2-AR agonist activated autocrine vascular endothelial growth factor (VEGF) in vascular endothelial cells (VECs), which then recognized and activated their VEGFR2 receptors. Plexin A1 is required for VEGF activation by VEGFR2 and affects vascular endothelial cell proliferation, migration and tube-forming capacity via activation of the janus kinase 2-signal transducer and activator of transcription 3 (JAK2- STAT3) signaling pathway in VECs to promote tumor angiogenesis [10]. *HIF-1α* is known to act on the

specific domain of the VEGF promoter region involved in angiogenesis [33]. In addition, expression of VEGF is up-regulated during hypoxia by *HIF-1α* [34] . Anxiety influences tumor angiogenesis through VEGF secretion, activating the b2-AR- *HIF-1α* pathway with prominent expression of pro-angiogenic growth factors VEGF, the tumor microenvironment has become "highly vascularized", favoring malignant tumor progression [10]. In the present study, a positive correlation was found between anxiety level and *HIF-1α*, our results support literature data that anxiety influences the *HIF-1α* signalization pathway which is a transcription factor essential for VEGF expression and tumor progression. Further studies are needed to understand the molecular mechanism by which anxiety influences the preceding signaling pathways.

SBR grade, an influential prognostic factor in BC, has also been associated with cell proliferation, a consistent indicator of response to chemotherapy. Determining an association between SBR grade and responsiveness would be clinically useful [35]. Hao et al. [36] in a study of glioma, found that anxiety was positively correlated with an increase in disease grade. Another study by Bergerot et al. [37] found that most women with high-grade neoplasms suffered of anxiety. Smith et al. [38] noted that there is an influence been anxiety and several other cancer factors, including grade. In the present study, we also found a correlation between anxiety and BC SBR grade. Our results are in line with previous data in the literature showing an association between SBR grade and anxiety. More studies are needed to understand all the molecular mechanisms between psychological changes, notably anxiety, and BC grade.

LN status is crucial for predicting clinical classification and treatment decisions in BC early-stage, and may be the main indicator of locoregional recurrence [39]. The analysis of biomarker *Ki-67*, to predict LN metastases is now routinely performed by IHC, which correlates fairly well [40]. It has been reported that *Ki-67* expression may be associated with LN [41]. Another study found that high levels of *Ki-67* were positively correlated with a higher incidence of LN metastasis, including the number of LN containing tumor cells and the rate of LN metastasis [42]. Data from previous studies agree with our results, which show that *Ki-67* is correlated with LN count.

A large number of studies have been carried out on the relationship between inflammation and cancer. It is well recognized that inflammatory diseases can increase the risk of cancer development during tumor initiation, promotion, progression and metastasis. *IL-6*, one of the best characterized pro-tumorigenic cytokines, has been extensively studied due to its central role in physiological and pathological processes. *IL-6* is also elevated in many solid tumors, including BC, which is linked to poor prognosis and metastasis [43]. A study has shown that Il-6 is a good prognostic factor and has been reported as a marker of favorable outcome in BC [44]. Another study has shown that inflammatory pathways and genes are strongly upregulated by several different oncogenes including *HER2* and are essential to their ability to transform [45]. Our results showed that *IL-6* was not associated with *HER2* which agrees with the results of Milovanović et al. [46] suggesting that its prognostic effect was independent of *HER2*. In addition, no association was found with other parameters including ER, PR, LN count and *Ki-67*. The results obtained are in line with the study carried out by Baharun et al. [47].

Over the last three decades, numerous preclinical and clinical psychoneuroimmunological and neurobiological studies have been published to investigate in detail the mechanisms underlying the links between stress and cancer. In addition, preclinical studies have shown that interventions that reduce the transmission of stress-related signals from the nervous system to peripheral tissues reduce the incidence of cancer, attenuate cancer growth, inhibit the development of metastases and enhance the effects of oncological therapy [48]. During acute stress, the SAM axis activates the release of norepinephrine, commonly known as the "fight or flight" response. Following activation of the SAM axis, the HPA axis releases endocrine glucocorticoids, i.e. cortisol. Activation of cortisol by the HPA axis activates immune cells, including inflammatory cytokines (e.g. *IL-6*) to cope with the immediately perceived stressor, which then influences the maintenance of health or vulnerability to disease [49]. The effect of stress and stressors on *IL-6* has been studied in a variety of ways and significant changes in *IL-6* induced by stress in plasma or serum can be distinguished [9]. Our results demonstrate that there is no correlation between *IL-6* and stress, as well as anxiety. A study by Jehn et al. [50] concluded there was no association between *IL-6* and anxiety in metastatic BC. A meta-analysis showed that chronic stressors had no relationship with quantitative measures of the immune system [51]. Another study showed that *IL-6* levels in BC patients were all higher in the stressed group, but not significantly so [52]. These data support our findings.

In the last few years, obesity has been identified as a risk factor for the development of BC, and has been associated with a poor outcome [53]. The molecular mechanisms involved are unclear. Systemic leptin levels increase in obese individuals (BMI, greater than 25) and leptin stimulates BC cell growth in vitro [54]. It has been demonstrated in vitro that leptin, which increases in the circulation in proportion to body fat reserves, promotes the growth of BC cells. Animal models have also identified leptin as an important factor in the development of mammary tumors [53]. A recent study showed that leptin transactivates *HER2*/neu and promotes the growth of BC cells overexpressing *HER2*/neu, and its overexpression in breast tumors is associated with poor prognosis and invasive BC [55]. Studies carried out by Babu et al. and Biglia et al. [20, 56] found that BMI correlated with BC size. Another study by Kaviani et al. [57] found that obesity was correlated with the presence of metastases in axillary lymph nodes. Our results are in line with previous data in the literature that BMI is correlated with tumor size and lymph node count. Further studies are needed to understand the molecular mechanism and the molecules involved.

HIF-1α expression in BC is associated with more aggressive disease, as HIF target genes may promote cancer

cell survival in the hypoxic tumor microenvironment by regulating processes such as glycolysis, angiogenesis and metastasis. We still don't understand how growth factor signaling modulates the cellular response to hypoxia, nor what the implications are for *HER2*-positive BC [58]. Dong et al. [59] found that *HIF-1α* expression was elevated and correlated with *HER2*-negative BC. Another study by The Cancer Genome Atlas Network showed that high expression of *HIF-1α* target genes is commonly observed in *HER2*-negative BC [59]. Chaturvedi et al. [60] also found that the signaling loop of *HIF-1α* is expressed in the triple-negative type. In the present study, we found a significant negative association between *HIF-1α* and *HER2* (*HIF-1α* concentrations were higher in *HER2*-negative molecular types). These data agree with the results of previous studies in the literature. Further research is required to study the signaling pathways that link *HER2* and *HIF-1α*, to understand the molecular mechanisms between the two molecules.

In conclusion, our results show a positive correlation between anxiety and the transcription factor *HIF-1α*, which plays a very important role in tumor angiogenesis, and also with SBR grade, a major prognostic factor in BC, suggesting that anxiety may modulate tumor angiogenesis and increases tumor aggressiveness and grade.

Furthermore, no association was found between stress and other pathological and IHC parameters, suggest that stress does not impact these parameters. We also found no association between stress, anxiety and the inflammatory marker *IL-6*, suggesting that these psychological events do not influence *IL-6* expression. A negative association was also found between *HER2* and *HIF-1α*, which can probably be explained by a negative control mechanism between the *HER2* and *HIF-1α* signaling pathway.

Ki-67 was also found to be associated with the number of LN, stating that high expression of this protein significantly increases the risk of LN metastases in BC. Finally, an association was found between BMI and the number of lymph nodes, as well as with SBR grade, indicating that obesity plays a very important role in breast cancer progression, particularly on LN metastasis and SBR grade.

Author Contribution Statement

SY, ZT, AM: Literature search and preparation of manuscript, performance of techniques; NB: Recruitment of patients, collection of data from records, MM: help with analysis and interpretation of *IL-6* and *HIF-1α* results; ML: Interpretation of results of anxiety and psychological stress questionnaires and assistance with biostatistical analysis; SS: help with various techniques; TS: planning our work, help with various techniques, help with writing and guiding our work.

Acknowledgements

Funding statement

The work was funded by the University of Oran 1 Ahmed Ben Bella, as part of my post-PhD research work in our Biology of Development and Differentiation Laboratory.

Ethical Declaration

The study was approved by the ethical committee of Batna 2 University according to Algerian Ethics legislation and the experiment complies with Algerian legislation (R/U.B.2/2024). All participants gave their informed consent prior to the interview by completing questionnaires and agreeing to biological tests being carried out on their bodies.

Statement on data availability

Data supporting the results of this study are available from the corresponding author, [SY], on reasonable request.

We would like to express our sincere gratitude to all the medical staff and paramedics for their invaluable assistance in collecting biological samples and reviewing medical data. Their dedication and support were essential to the successful completion of this study.

Conflict of Interests

All the authors declare no conflict of interests.

References

1. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era: The role of histological grade. Breast Cancer Res. 2010;12(4):207. https://doi.org/10.1186/bcr2607.
2. Benitez Fuentes JD, Morgan E, de Luna Aguilar A, Mafra A, Shah R, Giusti F, et al. Global stage distribution of breast cancer at diagnosis: A systematic review and meta-analysis. JAMA Oncol. 2024;10(1):71-8. https://doi.org/10.1001/jamaoncol.2023.4837.
3. Antonova L, Aronson K, Mueller CR. Stress and breast cancer: From epidemiology to molecular biology. Breast Cancer Res. 2011;13(2):208. https://doi.org/10.1186/bcr2836.
4. Pedersen BK. The diseasome of physical inactivity--and the role of myokines in muscle--fat cross talk. J Physiol. 2009;587(Pt 23):5559-68. https://doi.org/10.1113/jphysiol.2009.179515.
5. Nielsen NR, Zhang ZF, Kristensen TS, Netterstrøm B, Schnohr P, Grønbaek M. Self reported stress and risk of breast cancer: Prospective cohort study. Bmj. 2005;331(7516):548. https://doi.org/10.1136/bmj.38547.638183.06.
6. Obeid EI, Conzen SD. The role of adrenergic signaling in breast cancer biology. Cancer Biomark. 2013;13(3):161-9. https://doi.org/10.3233/cbm-130347.
7. Clark SM, Song C, Li X, Keegan AD, Tonelli LH. Cd8(+) t cells promote cytokine responses to stress. Cytokine. 2019;113:256-64. https://doi.org/10.1016/j.cyto.2018.07.015.
8. Reiche EM, Nunes SO, Morimoto HK. Stress, depression, the immune system, and cancer. Lancet Oncol. 2004;5(10):617-25. https://doi.org/10.1016/s1470-2045(04)01597-9.
9. Rohleder N, Aringer M, Boentert M. Role of interleukin-6 in stress, sleep, and fatigue. Ann N Y Acad Sci. 2012;1261:88-96. https://doi.org/10.1111/j.1749-6632.2012.06634.x.
10. Lu Y, Zhao H, Liu Y, Zuo Y, Xu Q, Liu L, et al. Chronic stress activates plexina1/vegfr2-jak2-stat3 in vascular endothelial cells to promote angiogenesis. Front Oncol. 2021;11:709057. https://doi.org/10.3389/fonc.2021.709057.
11. Chiriac VF, Baban A, Dumitrascu DL. Psychological

stress and breast cancer incidence: A systematic review. Clujul Med. 2018;91(1):18-26. https://doi.org/10.15386/cjmed-924.
12. Cungi C, Bouvard M, Rey M. Deux Echelles Breves Et Pratiques D'auto-Evaluation De La Communication Etde L'affirmation De Soi. Etude De Validation. Revue francophone de clinique comportementale et cognitive: Two brief and practical self-assessment scales for communication and assertiveness. Validation study. French-language journal of behavioral and cognitive clinics:1998;3(1):7-15.
13. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70. https://doi.org/10.1111/j.1600-0447.1983.tb09716.x.
14. Seno S, Akita M, Hsueh CL. A new method of the immunohistochemical detection of cellular antigens for light and electron microscopy. Histochemistry. 1989;91(6):449-54. https://doi.org/10.1007/bf00492514.
15. Cheang MC, Martin M, Nielsen TO, Prat A, Voduc D, Rodriguez-Lescure A, et al. Defining breast cancer intrinsic subtypes by quantitative receptor expression. Oncologist. 2015;20(5):474-82. https://doi.org/10.1634/theoncologist.2014-0372.
16. Dean-Colomb W, Esteva FJ. Her2-positive breast cancer: Herceptin and beyond. Eur J Cancer. 2008;44(18):2806-12. https://doi.org/10.1016/j.ejca.2008.09.013.
17. Schlam I, Swain SM. Her2-positive breast cancer and tyrosine kinase inhibitors: The time is now. NPJ Breast Cancer. 2021;7(1):56. https://doi.org/10.1038/s41523-021-00265-1.
18. Onitilo AA, Engel JM, Greenlee RT, Mukesh BN. Breast cancer subtypes based on er/pr and her2 expression: Comparison of clinicopathologic features and survival. Clin Med Res. 2009;7(1-2):4-13. https://doi.org/10.3121/cmr.2009.825.
19. Foulkes WD, Reis-Filho JS, Narod SA. Tumor size and survival in breast cancer--a reappraisal. Nat Rev Clin Oncol. 2010;7(6):348-53. https://doi.org/10.1038/nrclinonc.2010.39.
20. Govind Babu K, Anand A, Lakshmaiah KC, Lokanatha D, Jacob LA, Suresh Babu MC, et al. Correlation of bmi with breast cancer subtype and tumour size. Ecancermedicalscience. 2018;12:845. https://doi.org/10.3332/ecancer.2018.845.
21. Subramaniam S, Bhoo-Pathy N, Taib NA, Tan GH, See MH, Jamaris S, et al. Breast cancer outcomes as defined by the estrogen receptor, progesterone receptor, and human growth factor receptor-2 in a multi-ethnic asian country. World J Surg. 2015;39(10):2450-8. https://doi.org/10.1007/s00268-015-3133-2.
22. Nindrea RD, Aryandono T, Lazuardi L, Dwiprahasto I. Family history of breast cancer and breast cancer risk between malays ethnicity in malaysia and indonesia: A meta-analysis. Iran J Public Health. 2019;48(2):198-205.
23. Narod SA, Tung N, Lubinski J, Huzarski T, Robson M, Lynch HT, et al. A prior diagnosis of breast cancer is a risk factor for breast cancer in brca1 and brca2 carriers. Curr Oncol. 2014;21(2):64-8. https://doi.org/10.3747/co.21.1656.
24. Melvin JC, Wulaningsih W, Hana Z, Purushotham AD, Pinder SE, Fentiman I, et al. Family history of breast cancer and its association with disease severity and mortality. Cancer Med. 2016;5(5):942-9. https://doi.org/10.1002/cam4.648.
25. Fouhi ME, Benider A, Gaëtan KZA, Mesfioui A. Epidemiological and anatomopathological profile of breast cancer at the ibn rochd university hospital, casablanca. Pan Afr Med J. 2020;37:41. https://doi.org/10.11604/pamj.2020.37.41.21336.
26. Bhurosy T, Niu Z, Heckman CJ. Breastfeeding is possible: A systematic review on the feasibility and challenges of breastfeeding among breast cancer survivors of reproductive age. Ann Surg Oncol. 2021;28(7):3723-35. https://doi.org/10.1245/s10434-020-09094-1.
27. Scoccianti C, Key TJ, Anderson AS, Armaroli P, Berrino F, Cecchini M, et al. European code against cancer 4th edition: Breastfeeding and cancer. Cancer Epidemiol. 2015;39 Suppl 1:S101-6. https://doi.org/10.1016/j.canep.2014.12.007.
28. Garcia-Laguna G, Gerena-Cruz MF, Sánchez AY, Monroy-Gomez J, Dueñas Z. Chronic stress caused by maternal separation is a possible risk factor for the development of 7,12-dimethyl benzo anthracene-induced breast tumors in rats. Physiol Behav. 2021;235:113399. https://doi.org/10.1016/j.physbeh.2021.113399.
29. Monroe SM, Slavich GM. Psychological stressors: overview. Stress: Concepts, cognition, emotion, and behavior. 2016 Jan 1:109-15.
30. Daviu N, Bruchas MR, Moghaddam B, Sandi C, Beyeler A. Neurobiological links between stress and anxiety. Neurobiol Stress. 2019;11:100191. https://doi.org/10.1016/j.ynstr.2019.100191.
31. Koolhaas JM, Bartolomucci A, Buwalda B, de Boer SF, Flügge G, Korte SM, et al. Stress revisited: A critical evaluation of the stress concept. Neurosci Biobehav Rev. 2011;35(5):1291-301. https://doi.org/10.1016/j.neubiorev.2011.02.003.
32. Schenker R, Ciurea M, Stovicek P, Ciubara A, Schenker M, Marinescu I. Depression and anxiety - risk factors in the evolution of breast cancer in women. BRAIN Broad Research in Artificial Intelligence and Neuroscience. 2022;13:135-58. https://doi.org/10.18662/brain/13.1Sup1/309.
33. Sung KC, Kim KS, Lee S. Hypoxic regulation of vegf, hif-1(alpha) in coronary collaterals development. Korean J Intern Med. 2005;20(4):295-302. https://doi.org/10.3904/kjim.2005.20.4.295.
34. Saponaro C, Malfettone A, Ranieri G, Danza K, Simone G, Paradiso A, et al. Vegf, hif-1α expression and mvd as an angiogenic network in familial breast cancer. PLoS One. 2013;8(1):e53070. https://doi.org/10.1371/journal.pone.0053070.
35. Amat S, Penault-Llorca F, Cure H, Le Bouedëc G, Achard JL, Van Praagh I, et al. Scarff-bloom-richardson (sbr) grading: A pleiotropic marker of chemosensitivity in invasive ductal breast carcinomas treated by neoadjuvant chemotherapy. Int J Oncol. 2002;20(4):791-6.
36. Hao A, Huang J, Xu X. Anxiety and depression in glioma patients: Prevalence, risk factors, and their correlation with survival. Ir J Med Sci. 2021;190(3):1155-64. https://doi.org/10.1007/s11845-020-02374-5.
37. Bergerot CD, Clark KL, Nonino A, Waliany S, Buso MM, Loscalzo M. Course of distress, anxiety, and depression in hematological cancer patients: Association between gender and grade of neoplasm. Palliat Support Care. 2015;13(2):115-23. https://doi.org/10.1017/s1478951513000849.
38. Smith HR. Depression in cancer patients: Pathogenesis, implications and treatment (review). Oncol Lett. 2015;9(4):1509-14. https://doi.org/10.3892/ol.2015.2944.
39. Wang Q, Li B, Liu Z, Shang H, Jing H, Shao H, et al. Prediction model of axillary lymph node status using automated breast ultrasound (abus) and ki-67 status in early-stage breast cancer. BMC Cancer. 2022;22(1):929. https://doi.org/10.1186/s12885-022-10034-3.
40. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, her2 status, and prognosis of patients with luminal b breast cancer. J Natl Cancer Inst. 2009;101(10):736-50. https://doi.org/10.1093/jnci/djp082.
41. Lindfors H, Ihre Lundgren C, Zedenius J, Juhlin CC,

Shabo I. The clinical significance of lymph node ratio and ki-67 expression in papillary thyroid cancer. World J Surg. 2021;45(7):2155-64. https://doi.org/10.1007/s00268-021-06070-y.
42. Yin Y, Zeng K, Wu M, Ding Y, Zhao M, Chen Q. The levels of ki-67 positive are positively associated with lymph node metastasis in invasive ductal breast cancer. Cell Biochem Biophys. 2014;70(2):1145-51. https://doi.org/10.1007/s12013-014-0034-1.
43. Chen J, Wei Y, Yang W, Huang Q, Chen Y, Zeng K, et al. Il-6: The link between inflammation, immunity and breast cancer. Front Oncol. 2022;12:903800. https://doi.org/10.3389/fonc.2022.903800.
44. Yahia S, Tahari Z, Medjdoub A, Tahari FZ, Bessaih N, Messatfa M, et al. Expression profile of interleukin-6, 4-hydroxy-2-nonenal, and hypoxia-inducible factor 1-α in women with breast cancer and their association with clinicopathological parameters. Contemp Oncol (Pozn). 2023;27(1):14-21. https://doi.org/10.5114/wo.2023.127199.
45. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell. 2004;6(5):447-58. https://doi.org/10.1016/j.ccr.2004.09.028.
46. Milovanović J, Todorović-Raković N, Radulovic M. Interleukin-6 and interleukin-8 serum levels in prognosis of hormone-dependent breast cancer. Cytokine. 2019;118:93-8. https://doi.org/10.1016/j.cyto.2018.02.019.
47. Baharun BNB, Safuan S, Abdullah NA, Selvam K, Nor Hisham SR. The influence of macrophage polarisation status and il-6 in breast carcinoma invasion. Revista de Senología y Patología Mamaria. 2022;35(2):100-8. https://doi.org/https://doi.org/10.1016/j.senol.2021.11.002.
48. Mravec B, Tibensky M, Horvathova L. Stress and cancer. Part i: Mechanisms mediating the effect of stressors on cancer. J Neuroimmunol. 2020;346:577311. https://doi.org/10.1016/j.jneuroim.2020.577311.
49. Hulett JM, Abshire DA, Armer JM, Millspaugh R, Millspaugh J. Rural-urban differences in neuroimmune biomarkers and health status among women living with breast cancer. Cancer Nurs. 2021;44(4):323-32. https://doi.org/10.1097/ncc.0000000000000802.
50. Jehn CF, Flath B, Strux A, Krebs M, Possinger K, Pezzutto A, et al. Influence of age, performance status, cancer activity, and il-6 on anxiety and depression in patients with metastatic breast cancer. Breast Cancer Res Treat. 2012;136(3):789-94. https://doi.org/10.1007/s10549-012-2311-2.
51. Segerstrom SC, Miller GE. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. Psychol Bull. 2004;130(4):601-30. https://doi.org/10.1037/0033-2909.130.4.601.
52. Mundy-Bosse BL, Thornton LM, Yang HC, Andersen BL, Carson WE. Psychological stress is associated with altered levels of myeloid-derived suppressor cells in breast cancer patients. Cell Immunol. 2011;270(1):80-7. https://doi.org/10.1016/j.cellimm.2011.04.003.
53. Cleary MP, Grossmann ME, Ray A. Effect of obesity on breast cancer development. Vet Pathol. 2010;47(2):202-13. https://doi.org/10.1177/0300985809357753.
54. Sauter ER, Garofalo C, Hewett J, Hewett JE, Morelli C, Surmacz E. Leptin expression in breast nipple aspirate fluid (naf) and serum is influenced by body mass index (bmi) but not by the presence of breast cancer. Horm Metab Res. 2004;36(5):336-40. https://doi.org/10.1055/s-2004-814490.
55. Soma D, Kitayama J, Yamashita H, Miyato H, Ishikawa M, Nagawa H. Leptin augments proliferation of breast cancer cells via transactivation of her2. J Surg Res. 2008;149(1):9-14. https://doi.org/10.1016/j.jss.2007.10.012.
56. Biglia N, Peano E, Sgandurra P, Moggio G, Pecchio S, Maggiorotto F, et al. Body mass index (bmi) and breast cancer: Impact on tumor histopathologic features, cancer subtypes and recurrence rate in pre and postmenopausal women. Gynecol Endocrinol. 2013;29(3):263-7. https://doi.org/10.3109/09513590.2012.736559.
57. Kaviani A, Neishaboury M, Mohammadzadeh N, Ansari-Damavandi M, Jamei K. Effects of obesity on presentation of breast cancer, lymph node metastasis and patient survival: A retrospective review. Asian Pac J Cancer Prev. 2013;14(4):2225-9. https://doi.org/10.7314/apjcp.2013.14.4.2225.
58. Meyer HJ, Gundermann P, Höhn AK, Hamerla G, Surov A. Associations between whole tumor histogram analysis parameters derived from adc maps and expression of egfr, vegf, hif 1-alpha, her-2 and histone 3 in uterine cervical cancer. Magn Reson Imaging. 2019;57:68-74. https://doi.org/10.1016/j.mri.2018.10.016.
59. Dong M, Wan XB, Yuan ZY, Wei L, Fan XJ, Wang TT, et al. Low expression of beclin 1 and elevated expression of hif-1α refine distant metastasis risk and predict poor prognosis of er-positive, her2-negative breast cancer. Med Oncol. 2013;30(1):355. https://doi.org/10.1007/s12032-012-0355-0.
60. Chaturvedi P, Gilkes DM, Takano N, Semenza GL. Hypoxia-inducible factor-dependent signaling between triple-negative breast cancer cells and mesenchymal stem cells promotes macrophage recruitment. Proc Natl Acad Sci U S A. 2014;111(20):E2120-9. https://doi.org/10.1073/pnas.1406655111.