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Original Research Article

Immunohistochemical Expression of Hypoxia-Inducible Factor-1α in Triple Negative Breast Cancer

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Abstract

Triple-negative breast cancer is a malignant neoplasm characterized by absent expression of estrogen, progesterone and human epidermal growth factor-2 receptors. Hypoxia-inducible factor-1 alpha is up-regulated under hypoxia and associated with induction of angiogenesis resulting in proliferation, aggressive tumor phenotype and metastasis. Determination of the potential role of Hypoxia-inducible factor-1 alpha in the development and conventional clinicopathologic factors, and to demonstrate their prognostic relevance in Triple-negative breast cancer. A pro and retrospective study of 281 female patients with breast cancer. The fifty four triple-negative breast cancer formalin-fixed, paraffin-embedded Specimenspresented from all cases were immuno-stained by Hypoxia-inducible factor-1 alpha Immunohistochemical marker. The age groups distributed from 23-85 years old, mean age 51.23±11.26 years. Overall cases, triple negative breast cancer presented in 19.2%; most frequently younger,grade III, tumor size ≥ 2 cm, and positive lymph node metastasis or recurrent more than other subtypes. Hypoxia-inducible factor-1 alpha expression was strongly associated and significantly differentwith grade (p=0.001) ,lymph node metastasis (p<0.001) and Ki-67 expression (p=0.003). Detected Hypoxia-inducible factor-1 alpha expression has an important role in pathogenesis, drug resistance and in uncontrolled proliferation cells or as important conductor of the worst prognosis in triple negative-breast cancer.

Key words: Triple negative-breast cancer, HIF-1α and prognostic factors.

التعابير المناعية النسيجية الكيميائية للعامل المحرض على نقص الاكسده-١الفا في سرطان الثدي ثلاثي السلبية

الخلاصة

سرطان الثدي ثلاثي السلبية هو ورم خبيث الذي يتميز بغياب التعابير لمستقبلات هرمون الاستروجين والبروجسترون وعامل نمو البشرة البشري -٢. العامل المحرض على نقص الاكسده-١الفاالذي يتنظم تحت تاثير نقص الاوكسجين و يرتبط مع تحريض الاوعية الدموية مما يودي الى تكاثر الخلايا وعدوانيتها وأنتشار النمط الظاهري للورم. تحديد الدور المحتمل له في التطوير والعوامل السريرية المرضية التقليدية و اظهار اهمية التكهن في سرطان الثدي ثلاثي السلبية. هذه الدراسة مستقبلية ورجعية تضمنت ٢٨١ سيدة ممن يعانون من سرطان الثدي. كانت أربعة وخمسون من حالات سرطان الثدي ثلاثي السلبية والتي أخذت عينة من أنسجتها المحفوظة بالفورمالين والمطمورة بشمع البارافينو صبغت مناعيا باستخدام المؤشر المناعي النسيجي الكيميائي للعامل المحرض على نقص الاكسده-١الفا. الفئات العمرية وزعت بين٢٣ - ٨٥سنة , متوسط العمر ١١،٢٦ ± ٥١،٢٣سنة. أن جميع حالات سرطان الثدي ثلاثي السلبية تمثلت بنسبة ١٩،٢٪وغالبا ماتصيب الاعمار اليافعة والمرحلة الثالثة, و ذات حجم مساوي ٢ سم، وتكون إيجابية الانتشارفي العقدة الليمفاوية أو ان تكرارهااكثر من الانواع الفرعية الاخرى. وأن التعبير للعامل المحرض على نقص الاكسده-١الفاارتبط بقوة وبفروقات معنوية مع المرحلة (P =٠،٠٠١),والانتشار في العقدة الليمفاوية (P <٠،٠٠١) وتعبير Ki-٦٧ (P =٠،٠٠٣). الكشف عن تعبير العامل المحرض على نقص الاكسده-١الفاله دور هام في المرضية, ومقاومة الأدوية، وفي تحديد تكاثر الخلايا الغير مسيطر عليها او ان يكون موصلا هاما في التطور الأسوأ للمرضى الذين يعانون سرطان الثدي ثلاثي السلبية.

الكلمات المفتاحية:الثلاثي سرطان الثدي السلبي،العامل المحرض على نقص الاكسده−١الفا والعوامل النذير.

Introduction

Out of the 26 different types of cancer, breast cancer is the most common in women with approximately 1.38 million new cases worldwide, which corresponds to 23% of the total cases and 14% of cancer death. In Iraq it constitutes 19.59% of the total cancer cases and alone is accounted for 31% of all new cancer cases among females [1, 2, 3, 4, 5]. Breast cancer is heterogeneous with a variable prognosis depending on different histological and molecular characteristics, which influence patient prognosis and tumor behavior [6, 7].The mortality rates of breast cancer were decreasedbyearly diagnosisand hostile different approachesof treatment protocols. The treatment approachesaredetermined by using prognostic and predictive parameters like the patient's age, pathological tumor grade, menstrual status, status of hormone receptors and human epidermal growth factor receptor 2 (HER2) [8].

Among all molecular subtypes, triple negative breast cancer (TNBC) has generated the greatest interest. Due to lack of expression of estrogen, progesterone and HER-2 receptor,specific targeted therapies are not effective, and chemotherapy is currently the only modality of the available systemic therapy [7, 9].TNBC tumors typically express of genes associated with proliferation, inhibition of apoptosis and tumor invasion [10].Therefore the documentation of new therapeutic goals and treatment tactics for TNBC is desperately needed [11]. It represents an important clinical subtype and overlap with basal-like group[9, 12, 13, 14]. Moreover, from a clinical point of view they are characterized by aggressive behavior and poorer prognosis. Standard therapy is associated with high relapse rates mainly localized to the lungs, central nervous system and lymph nodes and death within 5 years of diagnosis[15, 16, 17, 18].Numerous studies carried vision into risk factors related to TNBC were more likely to arise among women with a younger age, premenopausal, family history of BRCA1 (Breastcancergene1) mutation carriers and African American women than among women of other ethnicities[9, 19, 20,21].

Tumor hypoxia, in the last few years, molecular and in vivo studies leads to a series of biological changes that can promote enhanced malignancy growth primarily, metastatic behavior by the expression of a large number of metastasis related genes, maintenance of cancer stem cell, angiogenesis, glycolysis and other processes involved in cell proliferation and survival through the action of Hypoxia-inducible factor (HIF-1/HIF-2) driving several oxygen-sensitive signaling pathways[22, 23, 24,25]. It is related to poor response to therapy in various cancer types[26]. In addition, very low levels of oxygen induce the unfolded protein response to reduce metabolic demand under condition of severe energy "starvation"[27].

HIF-1 is a heterodimer transcription protein composed of an oxygen-labile α-subunit (HIF1-α) and a constitutively expressed HIF1- β subunit, also documented as aryl hydrocarbon receptor nuclear translocator (ARNT). While HIF-1β is constitutively expressed, HIF-1α levels are tightly regulated by rapid upregulation and degradation [25, 28]. HIF-1α is found almost in every tissue and highly expressed in many different tumors, including primary breast cancers, but infrequent in most normal tissues [29, 30]. HIF-1α stimulates the expression of many enzymes and other transcriptional genes[31]. The indicators of tumor hypoxia inTNBC are central fibrosis and necrosis [18]. The association between HIF-1α and the frequently triple negative familial breast cancer brings to special molecular features of TNBC Response to

endoplasmic reticulum stress, activation of unfolded protein response pathway, a factor that promotes degradation of HIF and blunts HIF-induced malignant cell behavior as (Sharp1) andhypoxia[32]. In addition, also associated with novel targeted therapy for TNBC, namely anti-HIF-1α chemotherapy and related agents. This is especially reasonable for given the frequent link of TNBC with central necrosis, a surrogate morphological marker for hypoxia [18].

This study was directed in the aim of finding out the percentage of TNBC in Iraqi breast cancer women and if there is any association between clinico-pathological parameters of the TNBC and other breast cancer subtypes also to detect any association between several clinical and pathological factorsof TNBC and overexpression of HIF-1α.

Materials and Methods

This prospective and retrospective study was conducted in the Department of Pathology in the College of Medicine-Babylon Universityinwhich281 female patients with primary breast cancer wereselected from data obtained from archives in the laboratories in AL- Hilla Teaching Hospital, Al-Sadr Teaching Hospital in Najafand AL-Hussein Medical City and Al-Sajad Private laboratory in Karbala during the period from November 2013 through April 2015. The patient's specimens were classified along with their immunohisto-chemical staining into two groups as 54cases of triple negativebreast cancer and 227 cases of other subtypes. Histological types and grade were divided according to the World Health Organization classification and modified Bloom-Richardson grade [٣٣]. The clinic-pathological variables comprising age, menopausalstatus, tumor sizeand site, type of surgery, recurrence, histological subtype, TNM Staging and grading system were estimated.

Tissue specimens:

Fifty four specimens of TNBC were maintained in 10% formalin and preceded routinely then embedded in paraffin blocks and new sections were made from each of the paraffin embedded blocks which included 4-5 μm thickness where one section made on a glass slide to be processed and stained with routine haematoxylin and eosin stain for the confirmed histopathological study and others made on positively charged slides to be subjected for the purpose of conducting IHC procedures to detect HIF-1α [34,36].

Immunohisto-chemical procedures:

Immunostaining was completed using the Avidin, Biotin Complex detection (ABC) system [٣٤]. The paraffin sections fixed on positively charged slides were back in an oven at 60 ˚C for overnight, then the sections were deparaffinized in pre-warmed xylene and Rehydrated through absolute, 90%, 80%, and 70% ethanol. The section then treated with retrieval solution low PH (6.0) (DakoK8005 Kit) by heating in water bath at 95^{o}C for 30 minutes (until boiling) to expose antigens before advance treatment. The whole technique below was achieved at room temperature and according to the instruction of the manufacturer (Universal Detection Kit, Dako, Denmark). Once washed in fresh phosphate buffer saline (PBS) between each steps belowfor 3-5 minutes, make a circle around sections byPAP pen, then the section incubated in enough hydrogen peroxide block for 5 minutes (Dako K8023 Kit).

The enough amount of primary antibody applied to cover specimens and incubated for 60 minutes using mouse anti human HIF-1α (1/200, HIF-1α [1A3] ab113642, Abcam, Cambridge, UK) before incubated with enough amount of Mouse (linker)(DakoK8023 Kit) for 10-20 minutes.Furthermore, afterapplied enough amount of horseradish peroxidase (HRP) conjugate for 20 minutes, followed by 5-10 minutes Incubation with the DAB(3,3'-Diaminobenzidinetetra hydrochloride) - containing Substrate Working Solution(Dako K8023 Kit).

Finally the slides Immersed in a bath of Myers haematoxylin and dehydrated with

graded alcohol 70%, 80%, 90%, absolute then cleared with xylene before the cover slip was put on slides mounted with Di-N-Butyl Phthalate in Xylene(DPX).

The negative control in which the Sections untreated with primary antibody and replaced by PBS were used. In addition, Non-TNBC sections as positive internal control and cervical lymph adenitis as positive external control were added in each run of TNBC sections to the precision and standardization of the elaborated IHC results.

Statistical analysis

Data were represented as numbers and percentages. The database was examined the association between different variables and the differences were associated for statistical significance by chi – square (X^2) test [35]. The difference was considered significant at P <0.05. The statistical analysis was performed using statistical package for the social sciences (SPSS) version 20.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

Clinico pathological parameters of patients:

The total number of 281 cases of breast cancer has been included in the present study, 19.20% (54 cases) of cases were TNBC. The age group distribution of the patients ranged from (23 to 85) years. The overall mean and slandered deviation of ages was 51.23±11.26 years. The most common age group involved was fifth to sixth decade, both in (32.4%). 55.6% of TNBC cases were below or equal the age of fifty years, while 50.7% of non- TNBC cases where more than fifty years oldas shown in Figure -1.

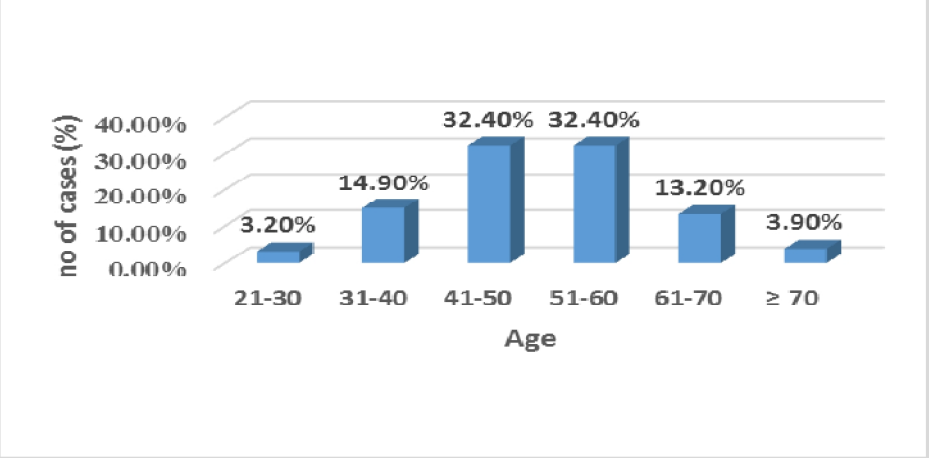

Figure 1: Distribution of Patients with breast cancer by Age groups

TNBC is mostly in the premenopausal women, 63% versus 58.6% in the other subtypes of breast cancer, TNBC was in the left breast in (55.6%) versus (52%) of others subtypes of breast cancer presented in left breast. Regarding the pathological features of the patients, the leading tumor type was invasive ductal carcinoma not otherwise specified (NOS) in TNBC (83.3% versus 78.4%) in other subtypes. A high percentage of TNBC 36 (66.7%) were found with grade III tumors while 70 (30.8%) in the other groups of breast cancer with significantly different (*P* <0.001), and according to the size of tumor 40 (74.1%) of TNBC were≥ 2 cm at presentation significantly different with 132 (58.2%) in the other subtypes of breast cancer *(P* = 0.008), a slightly higher proportion of patients with TNBC had T3 7 (13%) cases but different signification present with 10 (4.41%) in other groups (*P* = 0.015). 36 (66.7%) of TNBC had positive lymph node was higher than other group109 (48%)(*P* + 0.004), from 21 recurrent cases the TNBC presented in 6 cases (11.1%) while other subtypes presented in 15 cases (6.6%) table (1).

Table 1: Association of TNBC and Non-TNBC Types with Study Variables

Variable	Non-TNBC (%)	TNBC (%)	χ^2	*P value*
Age				
≤50 years	112 (49.3)	30 (55.6)	0.674	0.412
> 50 years	115 (50.7)	24 (44.4)		
Menopausal status	133 (58.6)	34 (63.0)		
	94 (41.4)	20 (37.0)	0.346	0.556
Premenopausal status				
Postmenopausal status				
Site of breast cancer			0.223	0.636
Right site	109(*48.0*)	24(44.4)		
Left site	118(52.0)	30 (55.6)		
Histological type				
DCIS	19 (8.4)	3 (5.6)		
NOS	178 (78.4)	45 (83.3)		
ILC	19 (8.4)	1 (1.9)	8.562	0.128
Medullary	6 (2.6)	3 (5.6)		
Mucinous	5 (2.2)	1 (1.9)		
Metaplastic	0 (0.0)	1 (1.9)		
Tumor grade				
Grade I	19 (8.4)	4 (7.4)	**24.699**	**<0.001***
Grade II	138 (60.8)	14 (25.9)		
Grade III	70 (30.8)	36 (66.7)		
Tumor size				
< 2 cm	62(27.3)	14 (25.9)	**9.688**	**0.008***
≥ 2 cm	132 (58.2)	40 (74.1)		
Not assessed	33 (14.5)	0 (0.0)		
T				
T-instu	3 (1.32)	0 (0.0)		
T1	60 (26.43)	15 (27.8)	**14.161**	
T2	117 (51.54)	31 (57.4)		**0.0١٥***
T3	10 (4.41)	7 (13.0)		
T4	4 (1.76)	1 (1.9)		
Not assessed	33(14.54)	٠(0.0)		
Lymph node metastasis				
Positive	109 (48.0)	36 (66.7)	**10.992**	**0.004***
Negative	85 (37.5)	18 (33.3)		
Not assessed	33 (14.5)	0 (0.0)		
Distant metastasis				
Positive (Mx)	172 (75.8)	41 (75.9)	**15.095**	**0.001***
Negative (M0)	22 (9.7)	13 (24.1)		
Not assessed	33 (14.5)	0 (0.0)		
Type of surgery				
Mastectomy	130 (57.3)	28 (51.9)	0.520	0.471
Lumpectomy	97 (42.7)	26 (48.1)		
Recurrence			1.279	0.258
Non-Recurrent	212 (93.4)	48 (88.9)		
Recurrent	15 (6.6)	6 (11.1)		

*p value ≤ 0.05 is significant

Immunohisto-chemical expression of HIF-1α and localization in TNBC:

The positive stained tumor cells were exhibited in a homogeneous pattern with dark brown punctate cytoplasmic and/or nuclear precipitate so used a semi-quantitative scale system. The percentage of positive cells were measured depends on the mean proportion of positive cells in five of high power fields of tumor cells in sections using the Olympus Light Microscope (magnification 40×).

The results were interpreted according to staining intensity and evaluating them according to the staining results for HIF-1α protein were classified as follows:0(No staining),+1 (Nuclear staining in less 1% of cells), +2 (Nuclear staining in 1–10% of cells and/or weak cytoplasmic staining), +3 (Nuclear staining in 10-50% of cells and/or distinct cytoplasmic staining), +4(Nuclear staining in more than 50% of cells and/or strong cytoplasmic staining) Figure (2). The results divided for further analysis into two group low expression included +1 or+2 and high expression presented as +3 or+4[36], it is detected in 38 cases (70.4 %) high expression, 16 cases (29.6%) showed low expression.

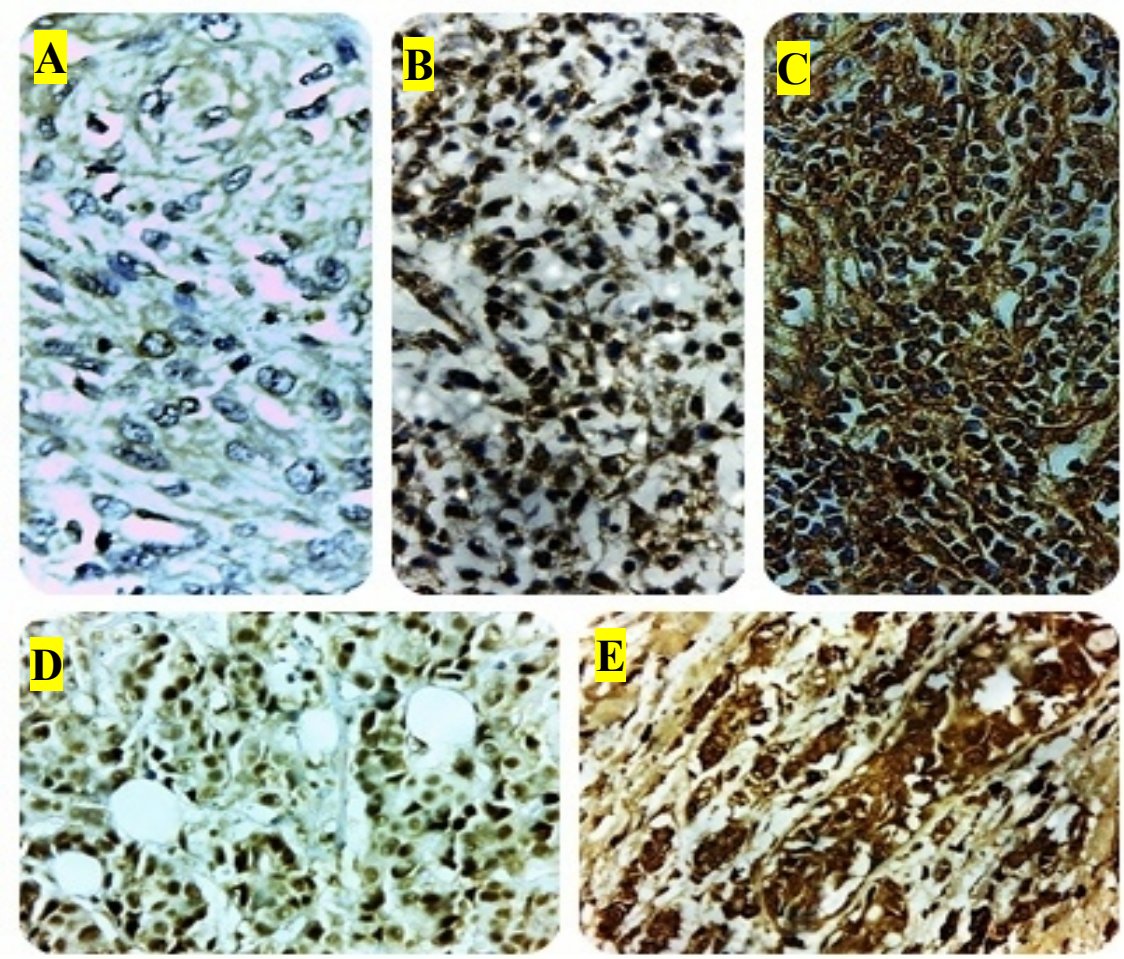

Figure 2:TNBC with (cytoplasmic and/or nuclear) expression of HIF-1α. (A) Negative expression; (B) Weak (1+) positive expression; (C) Moderate (2+) positive expression; (D) Strong (3+) positive expression; (E) Very strong (4+) positive expression (Immunohistochemistry x400).

Association between HIF-1α expression and Study Variables in TNBC:

There is no association between HIF-1α expressions in TNBC and the patient's age, menopausal status, tumor size, type of surgery, recurrence, T stage system, distance metastasis, histological subtype. There was a significant positive association between HIF-1α expression and nodal metastasis and histological grade and Ki-67 expression p value (P <0.001, P = 0.001 and P=0.003) respectively (Table 2).

Table 2: Association of HIF-1α with Study Variables in TNBC

Variable	HIF-1α		χ^2	*P* Value
	Low (%)	High (%)		
Age				
≤ 50 years	9 (56.3)	21 (55.3)	0.004	0.947
> 50 years	7 (43.7)	17 (44.7)		
Menopausal status				
	11 (68.8)	23 (60.5)	0.327	0.568
Premenopausal status	5 (31.2)	15 (39.5)		
Postmenopausal status				
Site of breast cancer				
	7 (43.8)	17 (44.7)	0.004	0.947
Right site	9 (56.2)	21 (55.3)		
Left site				
Histological type				
	1 (6.2)	2 (5.3)		
DCIS	11 (68.8)	34 (89.5)		
NOS	1 (6.2)	0 (0.0)	7.745	0.171
ILC	2 (12.5)	1 (2.6)		
Medullary	1 (6.2)	0 (0.0)		
Mucinous	0 (0.0)	1 (2.6)		
Metaplastic				
Tumor grade				
Grade 1	4 (25.0)	0 (0.0)		
Grade 2	6 (37.5)	8 (21.1)	**13.576**	**0.001***
Grade 3	6 (37.5)	30 (78.9)		
Tumor size				
< 2 cm	٥(٣١.٣)	٩(٢٣.٧)	٠.٣٣٦	0.٥٦٢
≥ 2 cm	١١ (٦٨.٧)	٢٩(٧6.3)		
T				
T-instu	0 (0.0)	0 (0.0)		
T1	5 (31.3)	10 (26.3)		
T2	10 (62.5)	21 (55.3)	1.413	0.703 [a]
T3	1 (6.3)	6 (15.8)		
T4	0 (0.0)	1 (2.6)		
Lymph node metastasis				
Positive	٤ (25.0)	٣٢(84.2)	١٧.٧٦٣	**<0.001***
Negative	١٢(75.0)	٦(15.8)		
Distant metastasis				
Positive	11 (68.8)	30 (81.6)	0.641	0.424
Negative	5 (31.2)	8 (18.4)		
Type of surgery				
Mastectomy	10 (68.8)	22 (57.9)	1.033	0.310
Another type of surgery	6 (31.2)	16 (42.1)		
Recurrence				
Non-Recurrent	14 (87.5)	34 (89.5)	0.044	0.833[a]
Recurrent	2 (12.5)	4 (10.5)		
Ki-67				
< 14%	10 (62.5)	٨ (*2*١.١)	**٨.٧٠4**	**0.00٣***
≥ 14%	6 (37.5)	٣٠(*7*٨.٩)		

*p value ≤ 0.05 is significant

[a:] Fisher Exact test

Discussion

The current study revealed that the age group of the breast cancer patients were ranging from 23-85 years, with a mean age and or standard deviation of 51.23±11.26 years, with 55.6% were younger than or equal 50 years. The peak age group frequently in the fifth to sixth decade of life was reported in this study. Our results consistent with most previous Iraqi researchers' reports in Bagdad Like Alwan NA [3],AL-Hashimi MMY & Wang XJ and AL-Khafaji HA *et al*, [4, 5].

In presented study compared TNBC rate with a proportion of١٥%–٢٠% in worldwide studies [20] which included 19.2% of the breast cancer patients. There were variable values as shown in Table (3) in comparison with this study may be due to variability in race, geographical area and IHC study.

Table 3: Rates of Patients with TNBC Typein Various Studies of Different Countries

Country	No. of cases	No. of TNBC	Percentage %	References:
Iraq Najaf	232	29	12.5%	[17]
USA	44704	6370	12.5%	[9]
China	1132	193	17.1%	[37]
Malaysia	996	175	17.6%	[15]
Lebanon	1834	170	9.3%	[38]
Iran	428	68	15.9%.	[39]
South Korea	276	97	35.1+%	[28]
Turkey	٧٥٢	204	27.1%	[10]

Numerous Asian and Western studied agree with the current study, that TNBC patients have a bad prognosis, in which were presented with younger age, premenopausal status and with frequently left breast affected and mastectomy specimen collection. In addition presented mostly as IDC (NOS) histological types and more recurrent with no significant difference than other IHC breast cancer types like that reported by Al-Khirsani&Yousif in Najaf [17], Tan *et al.,* in Malaysia [15], Payandeh*et al.*, in Iran [40], Lim Geok-Hoon*et al.,* in China and Dent *et al*., in Canada[41, 42].In contrast to our study,the relatively higher main age group and postmenopausal status reported by studies recorded by Abd-Elazeem *et al.,*in Egypt [43]and by Asleh-Aburaya *et al.,*in Palestinian Arab Kindred and Jewish population [16].

Moreover TNBC presented with increased association with large size, T3 stage, high grade (grade III) and lymph node or distant metastasis like reported by Al-Khirsani&Yousif in Najaf [17] Bauer *et al.,* in United States of America [9]. On the other hand the results in this study disagree with other researchers that suggested that no significantly different presented between above variables and TNBC like that reported by Kadivar*et al.,* in Iran [39], Yildiz*et al.,* in Turkey [10]**,** Tan *et al*., in Malaysia [15], Dent *et al*., in Canada and Hafft*yet al*.,in United States of America [42, 14].

In contrast, a study of a Western country was described that breast tumors are mostly older age, small size less lymph node involvement at the time of diagnosis[14, 42]. This result might be due to the Western countries with early detection programs more prevalent than our country and absence of efficient national breast cancer screening program in our country and high rate of malignant breast tumors in Iraq with poorly differentiated cells [3]. In addition, there were numerous theories have been

suggested to clarify this alteration, including non-modifiable risk factors such as (age at menarche,ethnicity and genetic difference), modifiable risk factors such as (socio-demographic information, parity, age at first pregnancy, breast feeding, anthropometric status, lack of physical activity, high fat diet and relative marriages which are not uncommon in our community) or due to use of advance procedure in prevention, diagnosis and treatment of breast cancer in United States or western regions. No-oneis completely satisfied and more researches are needed in this area to determine the predisposing factors in our patients [44]. The function of HIF-1α is under increasing survey by cancer researchers [45].

Van der Groep*et al.,*suggested that the amplified expression of HIF-1α in TNBC phenotype was estimated. In fact, this had been smartly demonstrated through the special expression of HIF-1α in Peoria-necrotic/ Peoria-fibrotic tumor cells in TNBC and BRCA1 mutated breast cancers[46]. Further Choi *et al.,*suggested that the expression of HIF-1α may be induced by hypoxia results in the loss of hormone receptors, which clarified as a characteristic of aggressive breast carcinomas revealing high proliferative activity, which tends to generate hypoxic areas where the expression of HIF-1α is increased [28].This fact agrees with the current study was clarified that 66.7% of whole women with TNBC were highly expressed in their histological sections.

However, as denoted in this study, the incidence rates of highly HIF-1α expressions were higher than what was reported byNajafi*et al*., in Iran, Yehia*et al*., inLebanon and Choi *et al.,*in South Korea by (55%, 35.5% and7.2% respectively). Beside that all suggested that the expression of HIF-1α highly in aggressive breast cancer phenotype)[47, 18, 28]. Moreover, there is a relatively higher rate of highly expressed HIF-1α in TNBC higher than in the present study that reported by Laurinavicius *et al.,* in northern Europe [7]. The current study in comparison with studies reported on breast cancer in general found commonly positive expressions rates of HIF-1α like that reported by the Egyptian Ibrahim *et al.,* and Chinese Lai *et al.,*researchers were found positive expressions rates as (64% and 79.5%, respectively) [26,48], the Taiwanese Huang Chih-Jen *et al*., The researcher was found30.2% higher positive HIF-1α expression 50 cases of breast cancer[49] and the Brazilian Tiezzi*et al.,*researcher found 50.7% positive HIF-1α expression from 75 cases of breast cancer [38].

Our study showed an insignificant association of HIF-1αexpression with clinic pathological variables like age, menopausal status, type of surgery, site of tumoror size and T stage system, histological type, local or regional recurrence and Distance metastasis of TNBC cases. This agrees with data reported in Western countries by Tiezzi*et al.,*Laurinavicius*et al*., andHelczynska[7, 26, 50] or in Africa by Ibrahim *et al.,* [26].

Otherdata show the controversy in association of tumor size withHIF-1αexpression the Choi *et al.*, and Kronblad*et al*., suggested significantly increased association [28, 51]. While Lia *et al*., found HIF-1α expression was inversely correlated with tumor size[48]. Also the Vleugel*et al*., suggested that breast cancer patients with positive HIF-1α expression have a worse disease free survival prognosis compared with patients with HIF-1α negative breast cancer, indicating the potential for HIF-1α as a therapeutic target and found that a significant correlation to incidence of local recurrence of breast cancer[52].

The frequency of high HIF-1αexpression is directly associated with poorer differentiated breast cancers than the corresponding well-differentiated lesions as mention in numerous studies previously [7, 28, 38, 45, 51, 52]. This

fact is also reflected in our study (78.9% high HIF-1αand in grade III). These findings suggest that HIF-1α expressed in the early stages of breast carcinogenesis [53]. On other hand Egyptian researchers have different result were reported high HIF-1α expression is directly associated with well to moderately differentiated breast cancers than the corresponding poorly-differentiated lesions [26].

The relation between Lymph Nodes metastasis and HIF-1αexpression also debate. We could find a significant association betweenHIF-1αand Lymph Nodes metastasis and this is similar to studies reported in Korea that presented an evidence for a possible role of HIF-1α as regulator of tumor-associated lymph angiogenesis in human breast cancer and emphasizes the promising status of HIF-1α as a therapeutic target against tumor progression and metastasis[28] and agree with numerous data reported previously [26, 38, 50]. Lia et *al.,* found inversely correlated with lymph nodes metastasis [48].

The presented study agree with suggestion thatHIF-1α expression play role in prognosis of breast cancer and closely related to the proliferation rate [53]. High levels of HIF-1α expression were also associated with proliferation markers Ki-67 and cyclin A levels that reported by Bos*et al*., and Kronblad*et al*., [53, 51].This study suggests that increased levels of HIF-1αwere statistically significantly associated with increased expression of Ki-67in TNBC this agree with the fact that the TNBC and HIF-1αboth associated with up-regulation of number of unfolded protein associated with hypoxia and increased population of stem or stem-like cancer cells and also expressed numerous proliferating genes [11, 18, 26, 28]. On the other hand, this study, which disagrees with other previous studies like that reported in Iran by Najafi*et al.,* [47] and by Laurinavicius*et al.,*in northern Europe [7] were found, no relation betweenHIF-1αand Ki-67 expression in breast cancer.

Conclusion

The percentage of TNBC in Iraqi women is accounted 19.2% of all breast cancer cases when existed as a bad prognostic type of breast cancer. HIF-1α is the hypoxia responsive transcription factor that involved in breast carcinogenesis. The expression of it in TNBC frequently high was within the universal range detected by others. The expression of HIF-1αin TNBC showed a significant direct association with the grade of tumor and lymph node metastasis with no relation to the age, menopausal status, site, histological type, and size, and distance metastasis, type of surgery and tumor recurrence. The study presented a significant direct association between The HIF-1αand Ki-67 IHC expression in TNBC. This may play important role in prognosis and in detection of new treatment protocol later.

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