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Cross sectional study of p53 immunohistochemical expression of HER2-positive and negative breast cancer patients

Alaa Talib Dawod
Faculty of Sciences, University of Kufa, Najaf, Iraq
Email: ella.talib1@gmail.com

Ali Hassan Abood
Faculty of Sciences, University of Kufa, Najaf, Iraq
Email: aliha.alkhafaji@uokufa.edu.iq

***Abstract*---**The current study included forty Iraqi female patients suffered from breast carcinoma were age varied from 34-75 years old, diagnosed through the period from 19 January 2019 to 7 April 2021. In present study used paraffin-embedded of female breast carcinoma sections have been confirmed histological diagnosis such as tumor size, differentiation degree and types of invasive breast cancers after reviewing all slides by specialist pathologists from different centers in AL-Najaf province. Cross sectional study of immunohistochemical expression of biomarker protein P53 (P53) positive and negative females with invasive breast cancers that have been conducted in Middle Euphrates Unit for Cancer Researches / University of Kufa. The highest percentage of P53 over expression was (12.5%) at age (50-59), while the lowest percentage found in age less than 40 years. The results obtained from the present study showed that there was significant difference (p<0.05) between P53 overexpression and patient age and according to histological type appeared of P53ve+ (27.5% in IDC and was (2.5%) in ILC, and P53ve+ which had significant difference (p<0.05) between P53ve+and histopathological type. As for the results of the present study of P53 expression with grade, the highest percentage of P53 over-expression appeared in grade 11 (15%). Cases with negative for P53 increased significantly in grade I1 more than in grade I, and 111(p<0.05.), Immunohistochemical expression of biomarker P53, had significant differences(p value< 0.05) according to tumor grade. The present study according to HER-2 has been appeared 7.5 % P53 over-expression in positive HER-2, and 22.5% in negative HER-2.This biomarker p53 had significant difference(p<0.05) with positive and negative HER-2.

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***Keywords*---**breast cancer, HER2-positive negative, p53.

Introduction

Breast cancer (BC) is the most common malignant tumor in women and the leading cause of cancer-related death in both developed and developing countries. [Starek-Świechowicz *et al.,* 2021]. This disease represent the most common cancer in women worldwide, with 1.7 million new cases diagnosed each year [1]. The presence of specific cell surface receptors, such as the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2), divides this diverse disease into many molecular subgroups (HER-2) [2]. The cell cycle, including cell proliferation, cell survival, and apoptosis, is regulated by HER-2 and on each chromosome, normal cells have one copy of the HER-2 gene, breast cancer cells, on the other hand, may contain 25-50 copies, resulting in enhanced HER-2 protein expression and the amount of HER-2 receptors on the surface of tumor cells [3].

HER-2 mutations are found in 71 percent of breast cancer cases, and HER-2 accounts for 10-15% of all breast cancers and it is distinguished by the absence of ER-related genes, strong expression of genes on the human epidermal growth factor receptor 2 (HER-2), co-localization, and hence co-amplification, with another proto-oncogene GRB7, and high expression of proliferation-related genes and this type does not have a ligand, but it is a favored dimerization partner of the other three receptors in the family [4, 5]. When the receptors (homo/hetero-)dimerize, downstream tyrosine kinase signaling cascades are triggered, causing cell proliferation, migration, invasion, and survival [6].

The protein 53 (P53) is a nuclear phosphoprotein with a molecular mass of 53 kDa and wild-type P53 protein is found in a wide range of normal cells, but it has a very short half-life and hence is only present in minute levels [7]. P53 in its natural state functions as a tumor suppressor, but mutant P53 acts as a dominant transforming oncogene [8]. The human P53 gene is found on chromosome 17 in the short arm of the human genome (17p13.1) [9]. There is a link between breast cancer and genetic abnormalities in genes and target genes, which are frequently associated with single nucleotide polymorphisms (SNPs) and one such gene of particular interest is 14-3-3sigma [10], which was first discovered in squamous epithelium and shown to be down regulated in a small number of breast cancer cell lines and it was later discovered that is a direct transcriptional target for p53 and that it mediates the maintenance of a G2 [11,12].

Methods

Study sample

Only Iraqi female patients were included in the current study, diagnosed through the period from 19 January 2019 - 7 April 2021. The study included 40 female subjects that their age varied from 34-75 years old. The samples were collected from Al-Sadder hospital and other centers. They were collected from females with invasive breast cancer center in Al Najaf province.

Experiment design

The present study included 40 cases of female with invasive breast cancer divided into two different tissue types, patients with invasive ductal carcinoma HER-2 positive or negative and patients with invasive lobular carcinoma HER-2 positive or negative). These cases of patients have been tacked from laboratory of histopathology in Al-sadder teaching hospital and private laboratories .It has been studied through Forty formalin-fixed, paraffin-embedded female breast carcinoma sections, that has been confirmed the histological diagnosis such as the tumor size, differentiation degree and types of invasive breast cancers after reviewing all slides by specialist pathologists from different center in AL Najaf province and patients having metastatic breast carcinoma with HER2 / neu protein overexpression detected by immunohistochemistry (IHC) or amplification analyzed by fluorescence in situ hybridization (FISH). Cross sectional study of immunohistochemical expression of biomarkers p53 in HER2 positive or negative females with invasive breast cancers that have been conducted in Middle Euphrates Unit for Cancer Researches / University of Kufa.

Histological preparations

All samples fixed after removing them from females with invasive breast cancer by true cut biopsy, excisional biopsy, quardectomy or mastectomy in container contains 10% formalin (38% 100ml formalin in 900ml tap water) and then done series of processes in sequenced steps [13]. Tissue sections has been examined by specialist pathologists from different centers in AL Najaf province by microscope (Human Type) in (10x and 40x) magnification. The tissues diagnosed as malignant were 40 blocks. Histological grading and size of tumor were also documented for each case, forty blocks of malignant tissues were included in IHC.

Immuno-Histochemica (IHC) Method

Immunohistochemistry (IHC) is used in histology to detect the presence of specific protein markers that can assist with accurate tumor classification and diagnosis [14]. Many proteins shown to be highly up regulated in pathological states by immunohistochemistry are potential targets for therapies utilizing monoclonal antibodies, and for monoclonal antibody preparations, the absolute concentration of specific antibodies can be readily measured, and frequently forms the basis for making the required dilutions [15]. In general, any immunodetection is achieved in two steps, the first includes binding the antibodies with target antigen, and the second is detection and visualizing this binding which is usually done by enzyme chromogenic system [16, 17]. In present study used Monoclonal mouse Anti human P53 protein immunohistochemistry kit stain, Dako Autostainer company, Germany.

Microscopic examination

Compound light microscope has been used to study changes in staining cells of breast tissues labeled by the antibody p53 was present in tumor cell nuclei. Photos have been taken to visualize some of results using a light microscope supplied with Optika camera.

Statistical analysis

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 23.0, SPSS Inc, Chicago, Ill, USA). A *P* value less than 0.05 was considered significant and the results analyzed through analysis of variance followed by the Duncan test.

Results

The table (1) shows the expression of P53 in patient females suffered from invasive breast cancer and relation with different clinical pathological variables. According to age there were significant differences ($p< 0.05$). in positive and negative expression of P53 and between them. The highest positive percentage of immunohistochemical expression of P53 was in age group (50-59) as the number five of cases reached a percentage (12.5) % of the total percentage of infected cases, while the lowest positive percentage of P53 was in the age group (<40) as the number one of cases reached a percentage (2.5)%. The highest negative percentage of p53 was in age group (50-59) (14, 35%) and lowest negative percentage was in age group (<40) (1, 2.5%) (table 1).

The data's of histopathological type of P53 +ve was highest in IDC (11, 27.5%) and lowest was in ILC (1, 2.5%), while in IDC of P53-ve was (21, 52.5%) and in ILC of p53-ve was (7, 17.5%) and the P53 immunohistochemical biomarker expression had significant difference ($p<0.05$) with histopathological type (table 1). Table (1) shown express of P53 in invasive carcinoma according tumor grade, and the highest percentage of P53 +ve was (6, 15%) in grade (II) and the lowest rate was (2, 5%) in grade (I) and also the highest rate of P53 -ve was (15, 37.5%) in grade (II), and lowest rate was (5, 12.5%) and the P53 immunohistochemical biomarkers expression had significant differences (p value< 0.05) according to tumor grade (table 1).

According positive and negative HER-2 of P53+ve the highest rate was (9, 22.5%) in negative HER-2 and lowest rate was (3, 7.5%) in positive HER-2 and the P53 Immunohistochemical biomarkers expression had significant differences($p< 0.05$) with positive and negative HER-2 as shown in the table(1). The figure (1) shown HER-2 positive invasive ductal carcinoma and HER-2 positive invasive lobular carcinoma with complete strong positive membranous brown stain. The figure (2) shown P53 negative invasive ductal carcinoma of breast with complete not display reaction patterns. Wild-type p53 protein is rapidly degraded and the figure (3) shown P53 positive invasive ductal carcinoma of breast with complete display reaction patterns, and diffuse strong nuclear brown stain. The figures (4) and (5) shown P53 positive well differentiated invasive ductal carcinoma of breast , strong nuclear brown stain and weak nuclear brown stain respectively. The figure (6) shown P53 positive moderately differentiated invasive ductal carcinoma of breast, strong nuclear brown stain.

Table 1
Express of P53 in invasive breast carcinoma according to different clinicopathological variables

Clinicopathological variables	P53+ve No(%)	P53-ve No(%)
Age (years)		
<40	1(2.5) D,a	1(2.5) D,a
40-49	2 (5) C,b	8 (20) B,a
50-59	5(12.5) A,b	14(35) A,a
>60	4(10) B,a	5(12.5) C,a
Total	12(30) b	28 (70) a
Histopathological type		
Invasive ductal carcinoma IDC	11(27.5)A,b	21(52.5)A,a
Invasive lobular carcinoma ILC	1(2.5)B,b	7(17.5)B,a
Total	12(30)b	28(70)a
Tumor grade		
I	2(5)B,b	8(20)B,a
II	6(15)A,b	15(37.5)A,a
III	4(10)C,a	5(12.5)C,a
Total	12(30)b	28(70)a
HER-2		
Positive	3(7.5)B,a	13(32.5)B,a
Negative	9(22.5)A,b	15(37.5)A,a
Total	12(30)b	28(70)a

*The different letters (Capital letters for column and small letters for row) refer to significant differences($P < 0.05$) while similar letters refer to non-significant differences for all biomarkers according to Duncan's test.

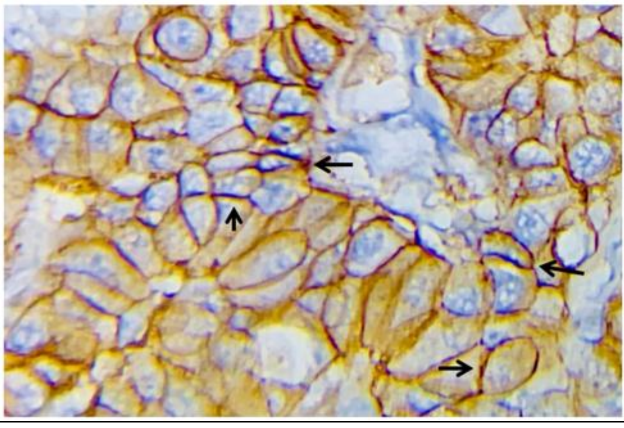


Figure (1): HER-2 positive invasive ductal carcinoma complete strong positive membranous stain IHC X 400.

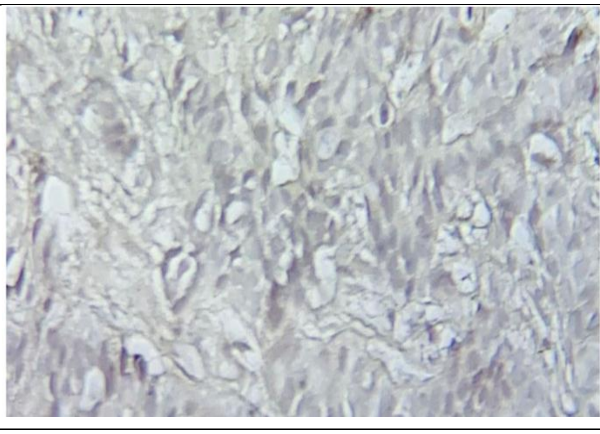


Figure (2): P53 negative invasive ductal carcinoma of breast IHC X 400.

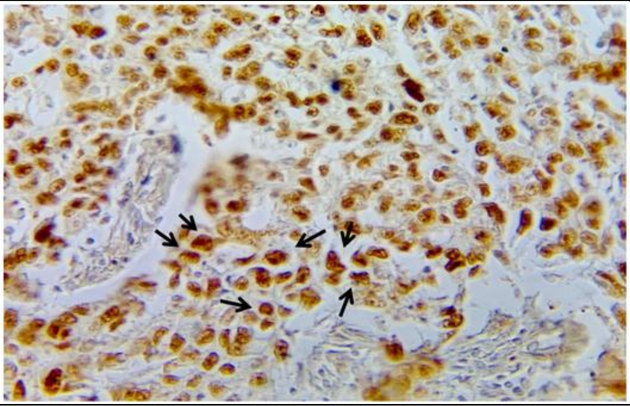
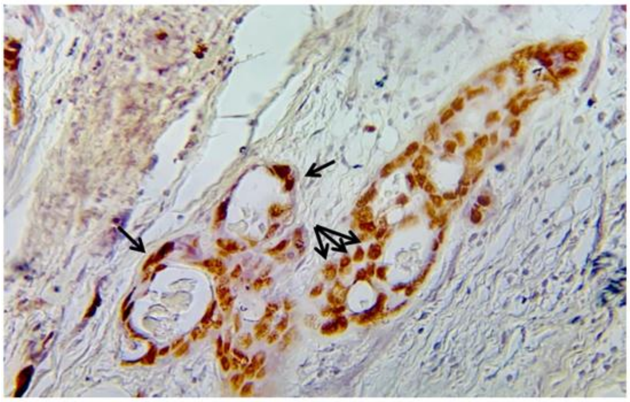
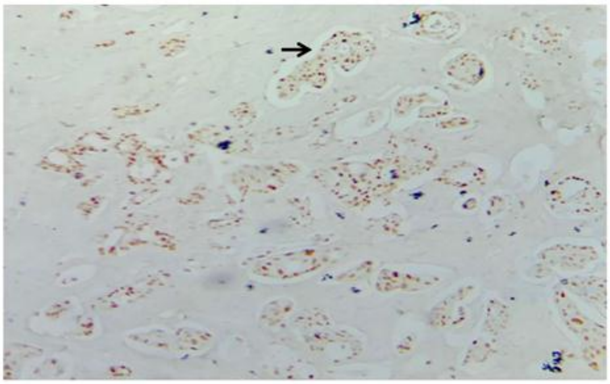


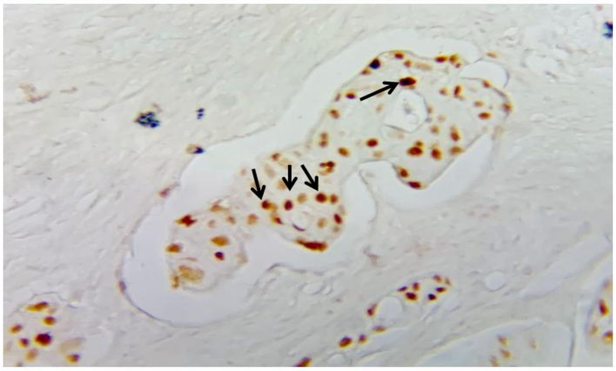
Figure (3): P53 positive well ductal carcinoma of breast diffuse strong nuclear stain IH X 400.



Figure(4): P53 positive well differentiated invasive ductal carcinoma of breast , strong nuclear stain IHC X 400.



Figure(5): P53 positive differentiated invasive ductal carcinoma of breast, weak nuclear stain IHC X 400.



Figure(6): P53 positive moderately differentiated invasive ductal carcinoma of breast ,strong nuclear stain IHC X 400.

Discussion

Coordination of transcription is one of the major responses to various stimuli from carcinogenic factors, which is programmed by P53 and ultimately suppresses tumor growth [18]. Loss of P53 function, primarily due to P53 mutation, has been discovered to be a common feature in the majority of human cancers, including breast cancer [19]. The current study's findings revealed that the fifth decade had the highest percentage of p53 positivity (12.5%), with higher expression of p53 being found in older patients age and the lowest rate of p53 positivity being found in younger age groups 2.5 percent, with a significant difference between p53 and age ($p<0.05$) as shown in table (1). This finding was similar to that of Levesque et al. (1998) and Pich et al. (2002), but it differed with Sierra et al. (1996), AL-Joudi et al. (2008), and Dash et al. (2021), who found no significant association between P53 and age[20, 21, 22, 23,24.

Knoop et al. (2001) found a strong association between P53+ and younger age, and AL Moundhri et al. (2003) found a substantial link between P53+ and younger age, suggesting that breast cancer among the youngest women has some biological distinctiveness [25,26]. This, in turn, may be due to a lack of samples taken from women with invasive breast cancer, as well as the random selection of samples used. Furthermore, basal-like breast cancer accounts for 10% of all breast cancers and 56 to 85% of triple-negative (TN) cancers reinforcing the fact that breast cancer patients in developing countries are diagnosed at a relatively late stage [27]. This is due to a lack of public awareness and screening initiatives.

Table 1 shows that positive immunoexpression of P53 was detected in paraffin-embedded tissue of ductal carcinomas in 27.5 percent of cases. This result was higher than that reported by Albederi & Yassin (2012) (23%), but lower than that reported by Mohamed (2006) (30%) and Dash et al. (2021), who found that 61.3 percent of patients had p53 positive. As indicated in table, positive immunoexpression of p53 was found in 2.5 percent of infiltrating lobular carcinomas, a significant difference (p0.05) from the previous histological type [24, 28, 29]. While Middleton et al.(2000) found 48 percent P53 positivity for lobular carcinoma, the results demonstrated that lobular carcinoma can be further identified by its immunohistochemical profile, which can be discriminated morphologically [30]. And, according to Arpino et al. (2004), no more than 5–10% of p53 positivity for lobular carcinoma, and according to Albederi & Yassin (2012), no more than 50% of p53 positivity for lobular carcinoma[31,32] . These distinctions Positive immunoexpression of P53 in histological types between the current findings and those of other research could indicate underlying biological mechanisms.

In this study, the maximum proportion of p53 positive occurred in grade II (15%), with the lowest proportion occurring in grade 1 (5%), and 10% in grade III. As demonstrated in the table, there was a statistically significant difference between p53 immunoexpression and tumor grade ($p<0.05$) (table 1). These results were similar to those discovered by Mhjoub et al (1999), Al-Joudi et al. (2002) and Orucevic et al.(2002) [33,34,35], on the other hand, found that the frequency of p53 mutation is directly correlated with the grade of breast cancers, i.e. higher expression is detected in higher grade. Pich et al.(2000) and Lacroix et al. (2006) found no significant link between p53 and breast cancer grade [36,37].

There are few research on the predictive value of these markers in poor nations, especially in the Arab world. In the current study, total HER-2/neu positivity was reported in 14 cases (40%) and total P53 positivity was reported in 12 cases (30%). Out of the forty cases, the highest percentage of P53 positivity was seen in HER-2-ve 9 cases (22.5%), while in HER-2+ve 3 cases (7.5%), with a significant difference ($p<0.05$) between P53 and their different expressions of HER-2 as shown in the table (1). Breast cancer is a diverse illness with a variety of biochemical subgroups, according to past research and TP53 mutation frequency varies by breast cancer molecular subtype, with luminal tumors having a lower incidence than basal-like or HER-2-enriched tumors [38].

According to the present results that the sub type with invasive breast cancer may be either luminal A, P53 is mutated in 13% [39]. Among all IDC subtypes, luminal A IDC patients have the best survival rate, this is due to both slow growth of the tumors and availability of ER-targeting agents such as tamoxifen, fulvestrant or aromatase inhibitors [40].

HER-2 subtype in invasive breast cancer. In 71% of cases, the P53 gene is mutated [41]. HER-2-positive and basal-like tumor types are linked to increased P53 accumulation. Patients with both HER-2 and P53 positive tumors, as well as those with bigger tumors and positive lymph node status, had significantly lower overall survival. P53 positive was found to have significant correlations with tumor size and histological grade. Patients with HER-2 and P53 positive tumors had significantly lower disease-free and overall survival than patients with HER-2 and P53 negative tumors and HER-2 or P53 positive cancers [42]. Overexpression of HER-2 and p53 has been linked to breast cancer progression, implying that these genes may play a role in the early stages of breast carcinogenesis [43].

In terms of a common biopathological profile, there was a high association between HER-2/neu and P53 immunoexpression. These findings corroborated those of Knoop et al. (2001), Mansour et al. (2003), Yamashita et al. (2004), and Bansal et al. (2017), all of whom found a substantial association between HER-2/neu and P53 immunoexpression [44, 45, 46, 47]. Tsutsui et al. (2003), AL Moundhri et al. (2004) and Lu et al. (2008), on the other hand, concluded that there was no association between HER-2/neu and P53 expression, and that the prognostic importance of these two parameters remained independent of each other [48,49,50]. The expression of P53 and HER2/neu is frequently higher in this form, which has negative hormonal receptor expression [51].

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