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EVALUATION OF THE P53 GENE EXPRESSION IN BREAST CANCER IN RESPECT TO AGE, GRADE, STAGE AND LYMPH NODES

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Abstract

It is previously reported that cancer prognosis is affected by mutations of P53 gene. However, the prognostic significance of P53 mutated gene detection in breast cancer is a subject demanding numerous investigations in a view of numerous facts such as presence of different tumor subtypes, P53 positivity in early breast cancer, different overall survival and disease free period as well as variability of tumor response to chemotherapeutic agents and presence of primary resistance of tumor among patients whether same or different grade and stage. Hence, it is of interest to detect this mutated gene in our area and evaluate its relation to other prognostic factors in terms of age, stage, histological grade and lymph node status.

This study aimed to evaluate the relation of P53 mutated gene expression in female with breast cancer in respect to the other prognostic factors such as age, grade, stage and lymph node status.

Fifty female patients diagnosed as a breast cancer, underwent clinical and pathological staging (I,IIA,IIB,IIIA,IIIB,IIIC, and IV), histopathological grading (I,II,III). All patients underwent same surgical operation which was modified radical mastectomy and axillary dissection. All specimens were sent to histopathological study and P53 mutated gene detection study by using immunohistochemistry (IHC).

P53 mutated gene was detected by immunohistochemistry in 72% of patients with breast cancer. P53 positivity showed a statistically significant direct proportion to histological grade, stage and lymph node status. In addition, more P53 mutated gene expression was detected in younger patients (age group ≤49 years old) and this probably explaining more advance stage observed in this group in this study. All these data leads to a conclusion that the presence of mutated p53 gene is associated with worse prognosis.

This study detected P53 mutated gene by IHC for the first time in our region and showed a statically significant association between status of P53 gene mutations and the other prognostic factors such as age, histological grade, stage and lymph node status and consequently to tumor aggressiveness and thereafter to prognosis.

Introduction

The P53 was first tumor suppressor gene which was identified in 1979. It is located in short arm of chromosome 17. The role of P53 is abolishing and inhibition of the proliferation of abnormal cell[1]. Thus inhibiting neoplastic development as an anticancer through numerous suggested mechanisms such as inhibition of cell cycle, apoptosis regulators, DNA repair and inhibition of angiogenesis and metastasis[1].

It is considered that tumor suppresser gene P53 mutation is an essential step in growth and development of numerous malignant tumors[2,3]. Alteration in P53 gene has been identified in many cancers including breast one[4].

Mutation of the P53 gene can be by mutagens like radiation, chemical agents or viruses raising the possibility of uncontrolled cell division affecting the DNA binding domain resulting in making a protein that has no DNA binding activity but able to bind to actively wild type P53[5,6]. So that, the identification and manipulation of tumor suppresser gene P53 may lead to understanding of the actual association of P53 status with stage and grade of breast cancer[7].

Breast cancer is the third most common tumor in the world and effects more than one million patients each year in the world and it is considered as a main cause of mortality[8]. Breast cancer in females is considered as a common one in Arabs countries and its highest percentage in Egypt, Kuwait, Lebanon, Iraq, Oman and Jordan; 35.1% , 34.4%, 33%, 16.7 %, 15.6% and 14.2% respectively[9-14].

It has been reported that there is wide range of variation among populations in terms of incidence, mortality, speed of growth. It is highest in developed countries and lowest in developing ones[15]. Breast cancer is a common cause of mortality in Basra city[16].

The breast cancer prognosis depends on several factors like size of primary tumor, location of tumor, status of lymph node, number of lymph node involved by tumor, histological grading and staging, age of patient, estrogen and progesterone receptor status[17].

The presence of P53 mutation in aggressive breast cancer is demonstrated in early study raising the possibility that P53 status effect biological behavior and subsequently many researches support the relation between P53 mutation and poorer survival. This relation was confirmed in a comprehensive meta-analysis showing that the breast cancer prognosis is affected by mutations of P53[18&19]. However, the relation between clinical outcome of breast cancer and P53 mutation is a subject of numerous investigation and debate. In breast cancers, P53 is mutated in almost 30% of cases, with a higher frequency in some tumor subtypes. In some reports, mutation of P53 is considered as a good prognostic factor, while in others it is considered as a poor prognostic factor. These variable outcomes could be attributed to different tumor types included in these reports and to various regimens of treatment or way of detection[20]. Moreover, the chemotherapeutic agents and hormone therapies used for localized breast cancer show a good response and improvement of survival.

The same agents are currently used for patients with metastatic disease but with a poor response exhibiting primary resistance and less overall survival in comparison to localized disease proposing the need for better understanding of the prognostic factors of response to treatment (chemotherapy, hormone therapy, radiotherapy)[19,21&22]. Hence in this project, it is a good idea to bring this technique (P53 mutation by IHC) in Basra and studying the association of P53 mutations with other known prognostic factors. And we use SPSS statics program in this study.

The aim is to detect expression of mutated P53 gene in patients with breast cancer and to evaluate its relation to other prognostic factors such as age, stage, grade and lymph node status.

Patients and methods

This is a prospective study which was conducted in the department of surgery in Basrah General Hospital and Al-Sader Teaching hospital, over a period of time (from the first of March 2014 to March of 2016). In the current study, there were 50 female patients (their ages were ranging from 31 years to 75 years) and the mean age is 49 years.

All patients were diagnosed as breast cancer (34 cases left upper lateral quadrant tumor, 10 cases right upper lateral quadrant tumor, 5 cases right lower lateral quadrant tumor and 1 case left central tumor). Ultrasonographic study was done for all of these cases. The size of tumors were 5x4x2cm (26 cases), 3x3x2cm (12 cases), 4x4x3cm (6 cases), 6x4x3cm (4 cases) and 1x1x1 (2 cases). All patients, in this study, were presented with breast lump. In addition to breast lump, 2 patients presented with bloody nipple discharge and other 2 patients with mastalgia. Patients with positive axillary lymph node were 34 and with negative axillary lymph node were 16. All cases have invasive ductal carcinoma diagnosed preoperatively by cytological or histopathological studies as follow; fine needle aspiration cytology (FNAC) for 45 cases and excisional biopsy for other 5 cases. All the patients underwent same surgical procedure which was modified radical mastectomy and axillary dissection subjected to a uniform procedure for grading of specimens (I,II,III) where G I is well differentiated, G II is moderately differentiated and G III is poorly differentiated.

In grading we depend on the most commonly used system is Elston\Nottingham modification of bloom-Richardson system, based on (a) tumor tubule formation, (b) number of mitotic figures in most active areas, and (c) nuclear pleomorphism.

P53 immunostaining study performed as follow: a formalin fixed and paraffin embedded tissue section were dewaxed, rehydrated and incubated in 10 mM citrate buffer (pH 6.4) for 20 min. in order to restore antigenicity. Phosphate buffered saline (PBS) wash was used between each of the following steps. Unspecific protein binding of tissue section was blocked with 1% bovine serum albumin containing (PBS) for 30 minutes at 37◦C, then the slides were incubated over night with primary antibody P53 [CM-1 rabbit polyclonal in 1:20000 dilution and D07 murine monoclonal 1:500 dilution, both from DAKO]. Primary antibodies were revealed by a biotin–streptavidin–amplified detection system [Biogenex, SunRoman, USA] using naphtol phosphate and fast red as color substrate.

Slides were lightly counter stained with hematoxylin and mounted in Karser's glycerin- gelatin[20]. Two observers assessed Immunostaining.

P53 score was calculated as the number of cells with positively labeled nuclei divided by the total number of neoplastic cells. In each specimen, a minimum of 1000 neoplastic cells were analyzed[21]. The definition of Immunohistochemistry (IHC) refers to the process of detecting antigens (e.g., proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissue. All biopsies had been checked up in a uniform way at one oncological out-patient department (Al-Sader teaching Hospital). See Figure 1.

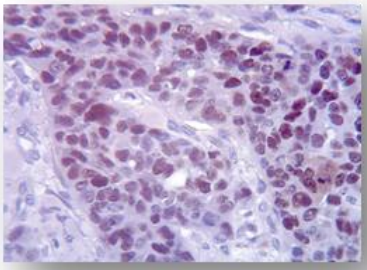

Figure 1: P53 mutated gene staining by immunohistochemical staining (IHC) to detect antigens in cells of a tissue section by using the principle of primary P53 antibody binding specifically to P53 antigens in breast tissue 400X.

Results

In the current study the mean age group was 49 years (31years-75years) and number of cases (24 case out of 50 cases) involved in breast cancer was in first group of age (31-49 years) representing (48%) and number of cases in second age group was 26 case (52%). Regarding the grade of disease 46 cases (92%) were in grade II and most of the cases were between stage IIA and stage IIIA. In 36 cases out of 50, p53 was positive (+ve) (72%) as shown in Table I.

Table I: Distribution of patients with breast cancer in terms of age, grade, stage and P53 mutation expression

Prognostic factors		No. of cases	Percentage %
Age	< 49	24	48%
	>49	26	52%
Grade	I	2	4%
	II	46	92%
	III	2	4%
Stage	I	0	0%
	IIA	22	44%
	IIB	10	20%
	IIIA	12	24%
	IIIB	6	12%
	IIIC	0	0%
	IV	0	0%
P53	+ve	36	72%
	-ve	14	28%
Total		50	100%

It was observed that P53 mutation detection by immunohistochemistry is significantly higher in patients with age group ≤49 years than patients with age group >49years (83.3%, 61.5% respectively) as shown in Table II.

It was demonstrated that detection of expression of P53 gene mutation was significantly increasing with increasing the grade; grade I (0%) , grade II (73.9%) and grade III (100%) reflecting the highest the grade the highest P53 expression.

It was found that there were statistically significant association between P53 mutation expression and the stage of tumor. The expression of P53 expression is increasing with

increasing the stage; stage I (0%), stage IIA (45.5%), stage IIB (80%), stage IIIA (100%), IIIB (100%) reflecting the highest the stage the highest P53 expression as shown in Table II.

Table II: Shows the percent of the prognostic factors in relation to the P53 mutation gene expression

Prognostic factors		No. of cases	P53		P value
			+Ve	-Ve	
Age	≤49 >49	24(48%) 26(52%)	20 (83.3%) 16 (61.5%)	4(16.7%) 10(38.5%)	0.010 sign.
Grade	I II III	2 46 2	0(0%) 34(73.9%) 2(100%)	2(4%) 12(26.1%) 0(0%)	0 .000 sign.
Stage	I IIA IIB IIIA IIIB IIIC IV	0 22 10 12 6 0 0	0(0%) 10(45.5%) 8(80%)) 12(100%) 6(100%) 0(0%) 0(0%)	0(0%) 12(54.5%) 2(20%) 0(0%) 0(0%) 0(0%) 0(0%)	0.015 sign.
Total		50	100%	100%	

In this study, it is found that there were statistically significant association between P53 mutation expression and the status of lymph node involvement showing that 28 cases (77.8%) with P53 expression +ve were associated with involvement of axillary lymph node by tumor whereas the rest 8 patients (22.2%) with P53 expression +ve were not associated with involvement of axillary lymph node by tumor as shown in Table III.

Table III: Shows the percent of the lymph node status in relation to the P53 mutation gene expression

p53	No. of +ve lymph nodes	NO. of -ve Lymph nodes	P value
Positive	28 77.8%	8 22.2%	0.022 sign.
Negative	6 42.9%	8 47.1%	

The current study shows there was no significant association between the age of the patients in in relation to the grade of breast cancer as shown in Table IV. But the association of age was significant to stage of breast cancer revealing significantly more patients in stage IIa and IIb in age group >49 years whereas patients in stage IIIa and IIIb were more in age group ≤49 as shown in Table V.

Table IV: The relation of the grade with Age

Age	No. of cases	Grade			P value
		I	II	III	
≤49	24	0 0%	22 44%	2 4%	0.134 in sign.
>49	26	2 4%	24 48%	0 0%	
Total	50	2	46	2	

Table V: The relation of the stage with Age

Age	No. of cases	Stage							P value
		I	IIa	IIb	IIIa	IIIb	IIIc	IV	
≤49	24	0 0%	6 12%	2 4%	12 24%	4 8%	0 0%	0 0%	0.000 sign.
>49	26	0 0%	16 32%	8 16%	0 0%	2 4%	0 0%	0 0%	
Total	50	0	22	10	12	6	0	0	

Discussion

In our series, P53 gene mutation expression was detected in 72% of patients with breast cancer. In one previous report P53 gene mutation expression is detected in (30%)[20]. Whereas in other previous report 70% of patients with breast cancer are positive for P53 gene[23]. This figure is variable in different previous studies as a result of different tumor subtypes and methods used in P53 gene mutation detection[20]. The commonest methods used are cDNA-based sequencing method and immunohistochemistry (IHC)[24]. It is reported that determining the P53 gene status in patients with breast cancer by using a cDNA-based sequencing method resulted in better prognostic information than IHC[24]. In current work, although IHC is recently admitted to our area, it was chosen because of availability of trained people and required equipment and materials for this method. In addition, it is of interest to mention that there were no previous report in our area about P53 gene detection and we are the first group working in determining the percent of patients with breast cancer having mutated P53 gene in Basra using IHC.

In current work, in order to evaluate the relation of P53 gene mutation to tumor aggressiveness and thus to prognosis, P53 gene mutation relation with other prognostic factors like age, stage, grade and lymph node status was studied. It was observed that P53 mutation detection by immunohistochemistry is significantly higher in patients with age group ≤49years than patients with age group >49years (83.3%, 61.5% respectively). This result is agreed upon it by work of many researchers that showed there is more P53 gene detection by IHC indicating that breast carcinoma in patients under 50 years is more aggressive than in patients 50 years and over[25,26]. However, many other workers reported that there is no significant relationship between age and P53[27-29].

The current study showed a direct proportion between status of P53 mutations and the histological grade. Percentage of P53 mutation expression increasing from no expression detected in grade I, to 73.9% in grade II (73.9%) till 100% in grade III. This result is

supported by previous other study that shows 7 % in grade I, 19 % in grade II and 43 % in grade III[25]. In addition, Sirvent et al. show that the P53 gene is over expressed revealing a direct statistically significant association to grade[23]. Many others support this observation in their work[30-32].

The current study showed that the expression of P53 gene is increasing with increasing the stage as follow; no expression detected in stage I, 45.5% in stage IIA, 80% in stage IIB, 100% in stage IIIA and 100% in stage IIIB. This observation comes consistent with results of previous reports who show that the highest P53 expression is associated with advanced stage[29,33,34]. In contrast, Many other workers state that increasing staging is statistically weakly associated to P53 positive gene expression or there is no significant correlation[35-37].

In this study, it is found that there were statistically significant association between P53 mutation expression and the status of lymph node involvement showing that 77.8% of patients with P53 expression positive were associated with involvement of axillary lymph node by tumor whereas 22.2% of patients with P53 expression positive were not associated with involvement of axillary lymph node by tumor. Many other previous reports show similar results that P53 positivity was also significantly related to the presence of axillary lymph node metastases[25,38,39]. Both features (P53 positivity and lymph node status) were also significantly more common in the younger age group[25]. While other articles report that no statistically significant association was found with axillary node status[23,40].

Having a direct relation between P53 gene mutation expression with other poor prognostic factors like advance stage, histological grade and axillary lymph node involvement reflects more tumor aggressiveness and hence poorer prognosis and survival. This conclusion comes consistent with results and conclusion of many others[24,25, 29].

In our series, although there was no significant relation between age and grade, we found that significantly earlier breast cancer (stage IIa and IIb) were in older patients whereas more advance breast cancer (stage IIIa and IIIb) were in younger patients. This could be explained by significantly more expression of P53 gene in younger age group showed above in current study. In other way around, presence of significantly more expression of P53 gene in younger age group comes consistent with advance aggressiveness of tumors in this group of patients and consequently their advance stage. This result and correlation comes in accord with some other researchers reporting an association of P53 expression with poorer outcomes in younger age group[25,41]. However, many patients with early breast cancer (early stage or grade or negative lymph node involvement) in our series showed P53 gene expression. Therefore, for better interpretation of the prognostic significance of P53 gene mutation in early breast cancer, long term follow up study is necessitated in collaboration with oncology unit examining the overall survival, disease free period and response to therapeutic treatment. In addition, validation of IHC method by other method such cDNA sequencing in future work is important to eliminate the possibility of false positive results[24]. Furthermore, it is reported that understanding of the importance of P53 expression in prognosis may need data of simultaneous estrogen (ER) and progesterone (PR) expression[41]. The cause for the relation between (ER and PR) and P53 is unidentified but possibly could indicate different types

of P53 mutations in tumors with or without ER and PR expression[41]. In current project, we have small-sized data about estrogen receptor (ER) and progesterone receptor (PR) expression and it is difficult to be linked with P53 gene expression. Hence, these data is not shown here. It is hoped to be completed later on for better understanding their relation to P53 gene expression and thereafter to prognosis.

In conclusion, This study showed a statically significant association between status of P53 mutations and the other prognostic factors (age, histological grade, stage and lymph node status) and thus to tumor aggressiveness and subsequently to prognosis. It was found that, in this sample of patients included in this study, significantly more advance breast (high stage) cancer in younger age group that could be explained by finding more expression of P53 gene in this group.

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